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FUNGOUS ROOT ROTS OF THE STRAWBERRY[1]

BY J. H. L. TRUSCOTT[2]

Abstract

This paper reports the results of two years of investigation at the Horticultural Experiment Station at Vineland, Ontario, on fungi associated with the root rot of strawberries. Roots from the field were collected periodically throughout two growing seasons and were studied by direct microscopical examination and by plating methods. Hundreds of isolates from diseased roots were tested by artificial inoculation of strawberry roots, and the primary parasites were further studied. These were classed in the following genera: *Pythium*, *Fusarium*, *Alternaria*, *Ramularia*, *Rhizoctonia*, *Verticillium* and *Cylindrocladium*. Microscopical examination revealed three additional forms, *Asterocystis*, a Plasmodiophoraceous fungus and the Phycomycetous mycorrhizal fungus, which are obligate parasites of Phycomycetous type. Some of the fungi occurred more frequently than others, and there were seasonal variations in the activity of most of them. A similar root flora was encountered in wild strawberry roots, so that most of these fungi are probably indigenous. Their relative importance can be determined only by several years of observation of field material.

Introduction

It is generally agreed among growers and agricultural field men in the Niagara Peninsula, Ontario, that strawberry yields have been decreasing during the past 20 years. According to their estimates present yields are from 50 to 75% less than those formerly obtained. Associated with losses in yield is the untimely death of plants or plant parts.

This condition is not peculiar to the Niagara Peninsula since somewhat the same general situation seems to exist in various parts of the United States and Great Britain. A review of the literature indicates that many factors have been considered by various workers to contribute to the reduced yields of strawberries. Of these the most important have been environmental factors and cultural methods (7), virus diseases (9, 17, 23), genetic aberrations (4, 8), various animal parasites such as nematodes (6) and aphis (5), and numerous fungous diseases of the leaves, crowns and roots.

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Contribution from the Department of Botany, University of Toronto, Toronto, Canada. This paper constitutes part of a thesis submitted to the Graduate School of the University of Toronto in partial fulfilment of the requirements for the degree of Doctor of Philosophy. Part of the work recorded here was done during the tenure of two scholarships from the National Research Council of Canada and the remainder as a co-operative project between the Department of Botany of the University of Toronto and the Horticultural Experiment Station at Vineland.

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The present work was undertaken at the Horticultural Experiment Station at Vineland, Ontario, in an effort to assess the importance of root-rotting fungi in the problem of strawberry degeneration. At this station great difficulty has been experienced in the strawberry breeding plots because many standard varieties and hybrid selections fail to maintain their initial level of production, owing to a marked loss of vigor by some plants and the death of many others. This has led in extreme cases to the loss of certain stocks. The most consistently occurring condition associated with unhealthy strawberry plants was badly rotted root systems, and consequently it was decided to investigate the role of parasitic fungi in bringing about this condition.

Methods

Many of the data presented herein were derived from thorough examination of roots from the field, such roots being collected periodically throughout two growing seasons. Each collection included the root systems of three to six plants. Root systems which were almost intact were obtained by careful digging and washing. Final washing was facilitated by the use of a camel's-hair brush with running water.

There is some difficulty in making direct microscopic examination of strawberry roots, so the procedure which was usually followed is given in detail. A strong light (a 120-watt bulb with reflector and condensing mirror) was necessary to permit examination of the smaller laterals and the edges of the larger roots. Further examination of the roots in the living condition required the use of material sectioned by hand in water-soaked pith. This was usually preferable to material which was crushed or dissected with fine needles. Larger roots were boiled in 5% potassium hydroxide solution to remove brown discolorations, and in Aman's lacto-phenol solution to which had been added a few drops of either cotton blue or acid fuchsin. This treatment cleared the material and stained most of the fungi present.

Some roots from each collection were planted on cornmeal agar in Petri dishes. The washed roots were rinsed in sterile water, the surplus water was blotted off, and then the roots were cut into pieces suitable for plating. Additional material for plating was obtained from roots which had been hand sectioned. When microscopic examination showed that a section contained a single desired fungus, its growth in the plate could be watched and consequently its isolation was greatly simplified. Examination of plated material was begun after 12 hr. and single hyphal tips were cut out of the agar when they appeared.

Direct microscopic examination of gross material was supplemented by studies of microtome sections. Masses of roots were imbedded in Parlodion after fixation in Carnoy's solution or formol acetic alcohol. Satisfactory staining was obtained by the use of Ehrlich's and Haidenhain's hematoxylin.

The value of direct microscopic examination of field material increases in proportion to the number of fungi that can be recognized thereby. The chief difficulty in this respect arose in attempting to differentiate between the

mycelia of the various imperfect fungi. Comparison of field material with that artificially inoculated with known fungi has made possible the recognition of most of the fungi commonly seen in field material. Certain exceptions will be dealt with later.

Since strawberry varieties do not breed true, seedlings are not suitable experimental material and large numbers of clean* plants were necessary for artificial inoculations. It was found that runner buds detached from the mother plants before the roots had broken through the swollen areas where they were formed, grew when planted in steam-sterilized soil. The writer's practice was to select those which were not in contact with the soil, and to wash them under the tap before planting. When growth conditions in the field were good nearly all of such runners could be used, but in hot, dry weather they seemed to be much less vigorous and the percentage of suitable material was reduced by one-half. In the writer's experimental material there was no evidence of fungous infection from the mother plant to the runner through the runner stem. The runner plant was healthy so far as one could judge by general appearances. Root systems were well developed, white in color and were actually free from fungous infection. The aboveground parts were of good color and vigor with no suggestion whatever of any virus diseases.

The parasitism of all of the culturable organisms herein described was tested by inoculations of clean plants in soil. A mixture of equal parts of moistened oat and barley seed, sterilized in flasks, was the medium used for increasing the fungi. From 10 to 20 gm. of such inoculum was distributed through steam-sterilized soil in six-inch pots before clean plants were transplanted into them. The plants were allowed to remain in the infested soil for periods varying from one to three weeks, when they were removed and the roots examined.

In such a study as this, one is soon overwhelmed with pure cultures of organisms of unknown parasitic capabilities, and tests of parasitism in artificially contaminated soil require much time and material. The following method of testing parasitism in a preliminary way proved very useful and was apparently reliable. Young runners with their roots just protruding may be had in large quantities during July, August and September in a favorable year. Such runners were washed under the tap and transferred to flasks containing moistened filter paper where they were inoculated with pieces of agar from plate cultures. The runners made considerable growth during the three or four days necessary for a test. This environment favors attack by most fungi, so that when they showed no indications of parasitism under these conditions they were discarded.

In addition to the fungi which may be artificially cultured there are commonly found in the roots several obligate parasites which no known plating method will reveal. Their effects on the plant can be determined only from the study of field material with the microscope. The criteria of their parasitism were the disappearance of the contents of host cells and the disintegration of tissues after invasion.

**Plants whose roots were free from parasitic fungi are referred to as "clean plants."*

Such evidence as is presented with respect to relative frequency and seasonal changes in the fungous flora of the roots was derived from direct observation of field material, supplemented by plating. Neither of the methods can be relied on to detect slight changes or small scattered infections by a given fungus. Hence, since the variations detectable by such methods are necessarily large and striking, they are probably significant in this important aspect of the complex root rot.

Materials

There was opportunity at the Horticultural Experiment Station, Vineland, Ontario, to make preliminary studies of the roots of many varieties of strawberries. The decadent variety Van Dyke appeared to be excellent experimental material, but the few plants extant at the beginning of the work died within a few months.

Premier was then chosen as the most suitable variety for further study. This was the source of most of the writer's isolations of fungi and it was the host for all parasitism tests.

Results

This preliminary elucidation of the roles of root-rotting fungi in the problem of strawberry degeneration involves so many fungi that it has been found most convenient to discuss each genus separately.

There is some variation in the treatment accorded certain of the fungi, particularly the genera *Pythium*, *Fusarium* and *Ramularia*. Our knowledge of Canadian forms of the genus *Pythium* is so inadequate that it was decided to investigate its mycology as well as its pathology in this instance. The genera *Fusarium* and *Ramularia*, on the other hand, have been studied in some detail in Canada. The genus *Fusarium* has received so much attention from taxonomists and pathologists that it was thought advisable to concentrate on less well known fungi. Because of the work of Walker (24) on the genus *Ramularia* in connection with the strawberry, the present study of this genus was confined to a comparison of its activities with those of other fungi.

No experiments were designed primarily to distinguish aboveground symptoms as a result of attack by the various fungi, and nothing of a distinctive character was noted in parasitism tests or in field material. In each instance there was stunting and in some cases the plant died. The stunting of the aboveground parts seemed to be the expression of a food and water shortage as a result of an extensively damaged root system.

(a) The Genus Pythium

Some hundreds of isolations of *Pythium* were made from strawberry roots and a few from other hosts growing in the same soil. The parasitic capabilities of certain quite different forms of the genus obtained from hosts growing in other regions were also tested. Most of the isolates were discarded either because of lack of virulence or because of duplication.

In vitro inoculations of the more virulent isolates of *Pythium* resulted in the complete destruction of the root system in 48 hr. The affected portions of the roots became brown in color and soft in texture. The mycelium entered the roots at any point, though there was an initial tendency to parasitize the regions near root tips. Entrance to the host was effected by means of a small penetration hypha usually produced from an appressorium at the end of a mycelial branch in contact with the host. Inside the host cell the penetration hypha immediately enlarged to the normal diameter of the mycelium and this process was repeated as it passed through succeeding cell walls. Host cells were rapidly killed. Though cortical tissues were damaged most commonly the fungus was able to penetrate the stele.

Artificial inoculations in soil resulted in extensive damage to the roots. Sometimes the damage was so severe that the plant died. Under such conditions the points of attack tended to be near pieces of inoculum scattered through the soil. Figs. 1 and 2 illustrate roots inoculated in soil with *Pythium* isolate "33–5." This was the only *Pythium* encountered which formed

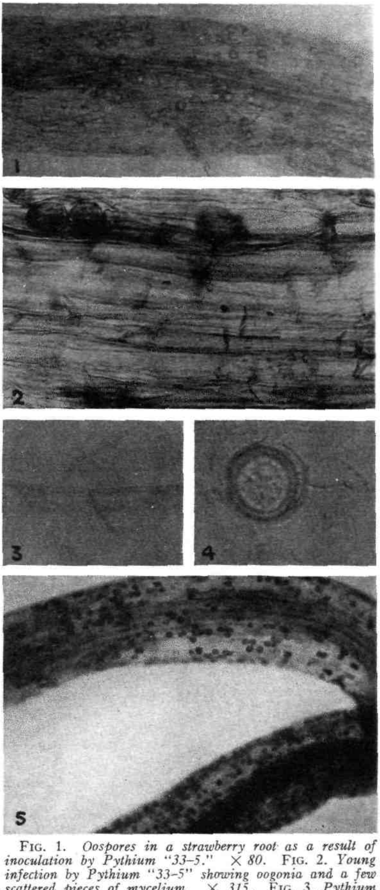

FIG. 1. *Oospores in a strawberry root as a result of inoculation by Pythium "33–5."* × *80.* FIG. 2. *Young infection by Pythium "33–5" showing oogonia and a few scattered pieces of mycelium.* × *315.* FIG. 3. *Pythium isolate "T" showing characteristic method of branching of the mycelium.* × *315.* FIG. 4. *Pythium isolate "T" showing oogonium, oospore and antheridium.* × *960. This type of antheridium is common to all of the Pythium forms described herein. The oospore wall is thicker than is usual.* FIG. 5. *Resting spores of Asterocystis.* × *52.*

oospores in strawberry roots. Such an infestation was not seen in field material. The same figures illustrate the difficulty of tracing mycelial threads in infested tissues. In very young invasions the mycelium is full of protoplasm and may be found easily. Somewhat later, however, it is seen only with great difficulty and is easily overlooked in field material.

Morphological studies of the parasitic isolates were undertaken in an attempt at specific identification. The life cycles are all incomplete in that none of the isolates were induced to form zoospores. This introduces difficulties in taxonomy, for the method of zoospore discharge is of major importance. However, the general characteristics of the isolates warrant placing them in the genus *Pythium*. They have all produced spherical bodies, which under appropriate conditions would be expected to form zoospores, but they consistently acted as conidia and germinated by germ tubes. Thus, according to Matthew's key (13, pp. 22–23), all belong in the *Sphaerosporangium* group in the section containing forms in which zoospore discharge is unknown. Sexual reproduction has been seen in some of the forms. With the data at hand it has not been possible to place them in any described species and it does not seem advisable to erect new species until it is more certain that the missing phases are unobtainable. After discarding obvious duplicates the number of parasitic isolates was reduced to nine, and these appeared to fall into four groups. One isolate from each group has been selected for description. Except where specified all observations were made from the fungi growing in cornmeal agar.

Group 1

Isolate T

Intramatrical mycelium yellowish *en masse;* capable of about 28 mm. radial extension in 24 hr. at room temperature; composed of rather straight main hyphae 7 to 9μ in diameter, side branches (2 to 7μ in diameter) which often make an angle of about 45° with the main branches (Fig. 3), and appressoria which are roughly oval in shape and measure 13 by 39μ.

Oogonia terminal or intercalary, smooth, subspherical; 18–26μ, average 22μ, in diameter. Antheridia 1 or 2 in number, monoclinous, usually clavate, making narrow, apical contact with the oogonium (Fig. 4). Sometimes cells are cut off on either side of the oogonium and apparently function as antheridia. Sometimes an oogonium functions as a conidium and germinates by a germ tube.

Oospore smooth, spherical; diameter usually about 3μ less than that of the oogonium bearing it; wall usually 1.5μ thick.

Isolated from tomato roots in the Vineland district in 1932. Weakly parasitic on the strawberry, strongly parasitic on tobacco, but inoculations of tomatoes have consistently failed. Similar forms have been isolated from the strawberry at Vineland.

Group 2

Isolate 39

Intramatrical mycelium colorless, capable of about 20 mm. radial extension in 24 hr. at room temperature; little aerial growth; composed of hyphae 2.5 to 8μ in diameter, and elongate appressoria which are often coiled twice.

Conidia terminal or intercalary, usually terminal; subglobular, 20–28μ in diameter.

Oogonia usually intercalary; smooth, lemon-shaped, 13 by 18μ to 18 by 20μ; often in series along a hypha.

Antheridia 1 or 2, usually 1; monoclinous, produced from close beside the oogonium; curved, clavate, making narrow apical contact with the oogonium; head measures 8 by 12μ; stalk often irregular in diameter. Sometimes functional, sometimes producing no penetration tube; oospores often formed when no antheridia are present.

Oospores do not fill the oogonium; diameter usually 3μ less than that of the oogonium bearing them; wall 1.5μ thick; oil globule 5μ diameter.

Isolated from Van Dyke strawberries at Vineland in 1932. Weakly parasitic on strawberry, strongly parasitic on tobacco.

Group 3

Isolate L

Intramatrical mycelium composed of rather straight main branches up to 8μ in diameter; side branches 2 to 8μ in diameter, and appressoria which are curved and swollen to about twice the diameter of the hyphae.

Conidia either terminal or intercalary, subglobular or lemon-shaped; 15 to 31μ, usually 26μ, diameter; tending to form in groups and early devoid of contents as a result of germination.

Oogonia seldom seen; the same size and shape as the conidia. Antheridia seldom seen and always appear to be functionless; clavate, curved, making narrow apical contact with the oogonium.

Oospores seldom seen; smooth, globular, usually filling the oogonium; wall 1.5μ thick; oil globule 13μ in diameter.

Isolated from lily bulbs at Vineland, 1932. Strongly parasitic on strawberry and tobacco. Isolate "36" from strawberries differs only in that conidia are produced much more abundantly and no sexual organs have been found.

Group 4

Isolate 30

Intramatrical mycelium composed of hyphae 3 to 9μ in diameter, the main branches of which tend to be straight. The mycelium coils when in contact with the glass in a Petri dish.

Conidia, in cornmeal agar, usually terminal and subglobular, 16–26μ, average 20μ, in diameter. In vegetable tissues they are more numerous, often irregularly shaped and reach a diameter of 30μ.

No sexual organs have been found.

Isolated from Van Dyke strawberry roots, Vineland, 1932. Parasitic on strawberry and tobacco. Isolate "33–5" appears to be a sexually active strain which was isolated from Premier strawberry in 1933. Isolate "*A*" differs from "30" in that it is much less parasitic on strawberry; isolated from apple roots in 1932.

These data show that Pythiums of several types are factors in the root rot of strawberries. They appear to be related to one another but they differ markedly in their parasitism on various hosts, in the presence or absence of certain organs, and in the readiness with which such organs are formed.

Certain peculiarities of these fungi must be understood if they are to be recognized in studies on strawberry root rot. It was noted before that it is only in very young infections that the mycelium can be readily seen and since resting spores are of rare occurrence there is soon little visual evidence of the organism which caused the rot. Plating must then be resorted to, and if the infection is not too old, the fungus can be obtained by using appropriate methods of isolation and suitable media. Single hyphal tips can be obtained rather readily when diseased roots are plated on cornmeal agar. Spore suspensions of fungi growing from roots in plates seldom contain culturable spores of *Pythium*, and lactic acid in media almost inhibits its growth.

During the course of these investigations the writer has had evidence that moisture conditions limit the parasitic activity of Pythiums. The growing season of 1932 was noteworthy for the abundance of rain in the Vineland district, and Pythiums were active in strawberry roots throughout the season. They were active during the earlier part of 1933 until drought set in, but for three months after that the writer was unable to obtain any evidence of their presence in this host. Rains began again in August, and within a week after the ground was thoroughly moistened Pythiums could be isolated at will. If it be true that moisture is the determining factor there will be a marked seasonal variation in the activities of *Pythium* species in districts where the spring and fall are typically wet and the summers relatively dry.

Owing to difficulties in the recognition of this group they have not always been given the attention which their parasitic capabilities warrant. A consideration of the host range from which the forms herein described were obtained, and of the hosts on which they have been proved parasitic, together with the fact that Pythiums seem to be present in all our cultivated soils, serves to emphasize the pathological importance of the genus. Pythiums have been reported on the strawberry from Australia by Adam and Pescott (1), from the United States of America by Plakidas (18) and from Great Britain by Wardlaw (25), but this appears to be the first report from Canada.

(b) The Genus Fusarium

Members of this group were constantly isolated during the course of this work. Most of the isolates were non-pathogenic but six forms which differed from each other in color and spore size were proved to be primary parasites of the strawberry. The following remarks are based on roots inoculated with a mixture of these six isolates.

Attack took place anywhere in the cortical region of the roots, the mycelium ramified freely intracellularly and death of invaded tissues rapidly ensued. Conidia were sometimes found in and on the tissues but more commonly only chlamydospores and mycelium were seen. Under conditions of heavy artificial inoculations in soil the stele was often invaded.

Members of this genus were consistently isolated throughout the growing seasons of 1932 and 1933. In drier periods they tended to be more apparent in platings of strawberry roots and this may indicate either the suppression of certain other fungi, such as Pythiums, or an actual increased activity of *Fusaria* during the warmer and drier periods.

Walker (24) reported the occurrence of *Fusaria* on the strawberry in Ontario and others have noted their presence in widely separated areas. Members of this genus are known to be present in nearly all cultivated soils.

(c) The Genus Ramularia

Walker (24) has done considerable work on fungi belonging to this genus in connection with strawberry root rot in Ontario, and Hildebrand's (10) work indicates that several forms of *Ramularia* are associated with root rots of other hosts in Ontario. Members of the genus were encountered from time to time during the course of this work, but inoculation experiments were confined to the use of a mixed culture of four isolates supplied by Dr. Hildebrand.

Soil inoculations resulted in extensively rotted roots. Microscopic examination of these roots revealed masses of mycelium and chlamydospores of a type so similar to those resulting from *Fusarium* inoculations that the writer is unable to distinguish the two genera on these bases.

The present work has confirmed the report that forms of *Ramularia* are primary parasites capable of causing extensive damage to strawberry roots.

(d) The Genus Alternaria

Alternaria appeared commonly in plate cultures from strawberry roots but none of the isolates were parasitic. A strain isolated from *Picea*, at Vineland, was obtained from Dr. Bailey, and in several pot tests it caused extensive root rot. In such inoculations the fungus was readily recognizable from the spores which were produced in some abundance in and on the root tissue. It has not been recognized in field material.

This form was a secondary parasite of aerial parts of *Picea* growing in the same region as the strawberries. The consistent failure of soil isolations of this fungus to show parasitism raises the question as to how commonly fungi exhibiting weak parasitism on aerial parts may become primary parasites when given the opportunity to affect roots. This is especially interesting in fungi as ubiquitous as *Alternaria*.

No previous record of an *Alternaria* causing root rot of the strawberry has been noted.

(e) The Genus Verticillium

Rudolph (20) and Thomas (21) reported the relation of *Verticillium* to a disease of the strawberry in America, the latter describing a specific disease which he called "Verticilliosis." The species known as *V. albo-atrum* has been reported very commonly in America and it is known to be present in the soils at Vineland. This is the species which Thomas encountered and he describes it as being much more easily isolated from the crown than from roots. In other hosts this species typically invades the stele.

Observations on strawberry material collected at Vineland gave no evidence of primary stelar infection either in the root or crown. In fact the crown was always remarkably free from invasion by any fungus until the plant was obviously nearly dead. *Verticillium* was not isolated from either roots or crowns. Since the fungus was known to be present in other hosts in the immediate vicinity, a culture derived from diseased raspberry canes was used for parasitism tests both in pots and in a single field test. Serious root rot resulted in both instances. This material was lost before a study was made of the steles and crowns.

Further work with this fungus is plainly necessary, since a parasitic form is present at Vineland and a similar form is known to cause a specific disease of the strawberry in other regions.

(f) The Genus Cylindrocladium

A single species of this genus was isolated during August and September of 1933. It has not been reported before as a parasite of strawberry in so far as the writer has been able to determine, and it does not appear to have been encountered commonly in Canada on any host. A brief description seems to be warranted.

An aerial growth of whitish, coarse mycelium is abundant in culture on cornmeal or potato-dextrose agar. The underside of plate cultures is characterized by a mahogany red color due mainly to concentric rings of large sclerotial masses. Conidiophores (Plate I, 1, 3 and 4) are produced extramatrically and are branched dichotomously. One-septate, cylindrical spores are borne in a cylindrical mass (Plate I, 1) and are cemented together with a substance which dissolves readily in water so that the spores are liberated as in Plate I, 4. A very characteristic feature of the conidiophore, and sometimes of other parts of the mycelium, is a sterile hypha (Plate I, 2, 3 and 4) bearing at its tip an enlarged, obovate cell. The abundant sclerotial masses are illustrated in Plate I, 5, 6, 8 and 9. This fungus conforms to the specifications of *Cylindrocladium scoparium* Morgan.

Pot inoculations with this fungus showed a more virulent type of parasitism on strawberry than was obtained with any other fungus with which the writer has worked. Death of the plant within a week was a common occurrence and none survived longer than two weeks. The whole root system was rapidly rotted and the leaves which were on the plant at the time of inoculation suddenly wilted as a result of the complete destruction of the roots.

The fungus can be identified with certainty in the host by the presence of the characteristic sclerotial masses which may be produced either extra- or intramatrically (Plate I, 8 and 9), and by the very curious mycelium which is shown in Plate I, 7. The method of branching is also characteristic in agar cultures and it can be identified there by that character alone.

This fungus was seen only during about three weeks in August and September of 1933, but during that time several roots gave practically pure cultures of it. Since its characters are so distinctive there is little chance that it was previously overlooked. If that be true, there was a well marked period of activity late in the summer after about two months of dry weather.

C. scoparium was first described as a saprophyte on the pods of *Gleditschia* and it is known to be the cause of a serious crown rot of roses. It must now be considered an important addition to the list of strawberry root parasites.

(g) The Genus Rhizoctonia

One of the fungi most commonly seen in strawberry roots during two years of observation is that shown in Plate II, 5. It was obtained in pure culture in August, 1933.

It grows readily in common laboratory media producing a grayish, granular colony in plate culture. The most obvious character is the production of monilioid bodies as shown in Plate II, 3. These do not behave as chains of spores but form sclerotial masses in host cells (Plate II, 1 and 4). Peyronel (16) has described fungi which he calls *Rhizoctonia* and they have the same cultural characters as this organism. The relationship appears to be quite definite. Another character which he noted is the tendency of the mycelium to form coils in host tissue. Plate II, 2, shows the strawberry fungus in root tissue and there is some tendency towards coiling.

In artificial inoculations of the strawberry this fungus is actively parasitic. Definite and extensive root rot is brought about. In field material there is no doubt that invaded cells are killed. When it was realized that Peyronel (16) had reported a similar organism in mycorrhizal relations with various hosts a careful canvass of that possibility was made. However, a check on the material available gave no evidence of mutualistic relationships between the strawberry and this fungus. Plate II, 1, illustrates the extent of the damage which was caused by this fungus.

Its period of activity was not easy to determine by direct microscopic examination of field material. The mycelium seems to be quite resistant to decay and may be found in quantities on previous years' roots. Many attempts at culturing such mycelium were unsuccessful and it is now assumed that it was dead. Positive evidence of activity was obtained in the fall of 1933 when the fungus was isolated readily. At that time it appeared in abundance in the field and remained active in potted field material kept out of doors, as late as December 20, though during that period the soil was frozen solidly several times. There is an interesting possibility that this fungus may be partially responsible for the death of strawberry plants during the dormant season.

This appears to be the first report of a *Rhizoctonia* parasitizing strawberry roots in Canada.

(h) The Genus Asterocystis

A single species of this genus was commonly found in strawberry roots. It is an obligate parasite and there is no known method of pure culture inoculations. It was studied in the living state in hand sections of strawberry roots mounted in water, and in lacto-phenol preparations.

Fig. 5 illustrates the appearance of a heavy infection of *Asterocystis* in stained material. Infection is confined to the outer cortical layer and can take place only by means of zoospores, since there is no mycelium. After penetration there develops in the host cell a globular or oval body which may function as a sporangium or develop into the characteristically rough-walled resting spore. If it is to function as a sporangium an exit tube is produced which punctures the host cell, and zoospores are liberated into the surrounding water. The writer has studied material described by Vanterpool (22) and can find no differences between the fungus he called *Asterocystis radicis* de Wild. and the form in strawberries.

Asterocystis is not likely to be mistaken for any other fungus because of the characteristic markings on its resting spores. It was constantly seen in an active condition throughout the wet summer of 1932 and again in the spring of 1933. Infections were sparse or lacking in specimens examined during the dry season of 1933. The necessity of free water for the dissemination of the zoospores accounts for the heavy infections in moist soil.

Fig. 5 gives an adequate idea of the amount of tissue that is often destroyed by this fungus. The loss of this storage tissue must affect the plant as a whole. The normal feeding habits of the roots may also be disturbed when the outer cortical layers are largely destroyed. There is also the possibility that cells killed by *Asterocystis* may facilitate the attack of weaker parasites which would be less successful in invading uninjured roots. The fungus seems to be present in most soils and to inhabit the roots of many plants. No previous report of this organism attacking strawberry has been found.

(i) Plasmodiophoraceous Fungi

Scattered infections of organisms belonging to the Plasmodiophoraceous group of Phycomycetes were seen frequently in strawberry roots. Material was not abundant enough for a complete study but the resting spores indicate a relationship to the group represented by *Spongospora*. Infection was always confined to the outer cortex and consisted of a solid spore mass or masses which might or might not fill the host cell. One form closely resembles *Polymyxa graminis* Ledingham (12) which was reported in wheat roots growing beside the strawberry plantations at Vineland. Several variations of spore arrangements were noted and this may indicate several organisms. This group depends on zoospores for the spread of infection and its occurrence in strawberry roots coincides with damp soil conditions.

(j) The Phycomycetous Mycorrhizal Fungus

An organism known as the Phycomycetous mycorrhizal fungus has been reported in the roots of many plants. There appears to be no previous Canadian record of this fungus in the strawberry, but its presence in this host has been noted by Jones (11) in the United States of America and by O'Brien and M'Naughton (14) in Great Britain.

Of the fungi identifiable under the microscope in field material, this one was the most conspicuous and the one most consistently present. As a result of observations made during two years and involving about 25 standard and seedling varieties, it may now be assumed that the root system of every mature strawberry plant at the Experiment Station, Vineland, is infected to a greater or less degree by this fungus.

This fungus has not been studied in detail in Canada in relation to the strawberry and descriptions of its life history in various hosts have lacked some phases which one expects in a fungus which appears to be a Phycomycete. Consequently it was desirable to make a study not only of its pathogenic capabilities but of its morphology and life cycle.

From the masses of mycelium sometimes seen both in and on strawberry roots and the apparent lack of host specificity, one would expect that the organism might be cultured. The following media were given thorough trial:—many of the common liquid and solid laboratory media, Ayers' purely protein media (3), several animal and vegetable fats and oils and various decoctions and tissue media from strawberry plants. Rootlets infested with the organism were sometimes used as sources of inoculum, but in every case other fungi and bacteria were present in sufficient numbers to prevent long-time observations. However, dissections of the roots made it possible to obtain quite large portions of mycelium free from host tissue and other fungi, and they were relatively free from bacteria. By occasional changes of the medium it was possible to keep such cultures for weeks, but no growth occurred in any of these media.

The lacto-phenol method is very satisfactory for spotting the fungus in roots during certain stages of its development but it is not well suited to bring out other stages, so this method should be supplemented by hand sections of living material or by dissection with needles. Sometimes direct observation of untreated roots shows extramatrical mycelium and some distortion of the tissues due to the abundance of the fungus within. For the finer details Parlodion sections were found to be satisfactory and in this report most of the observations made from such material were checked by means of hand sections of living tissues.

Penetration is effected by means of mycelium which may enter at any point in the younger cortex of either primary or lateral roots, the latter being most commonly attacked. The penetration hypha follows a tortuous course between and through the outer cortical cells (Plate IV, 1) and usually begins to branch and spread only when the inner regions are reached. The coarse mycelium spreads mainly through the intercellular spaces (Plate IV, 3 and 9) and tends to acquire the configuration of the intercellular spaces (Plate IV, 2).

The layers of cortical tissue next the stele are invaded and killed first, and for some time there is no discoloration of the outer part of the root. Sometimes the fungus spreads outwards as well as in a longitudinal direction so that most of the cortex of the root is occupied (Plate III, 1, 2, 3 and 4). More commonly, however, the outer layers are not invaded but they eventually die and the whole rootlet becomes brown in color. The death of the uninvaded outer cortex may be due to its being cut off from the stele by a layer of dead cortex but the death of cells seems to be progressive from the cells close to the fungus to those farther away and this suggests that the fungus may be implicated directly.

The structures produced from the mycelium in the strawberry have proved to be similar to those described in other hosts. The intercellular mycelium produces short lateral branches which enter the host cells and develop highly complex structures known as arbuscles (Plate IV, 4, 5, 6, 7 and 8). The type seen in Plate IV, 4, seems rather rare and it is doubtful whether it represents a stage in the development of all arbuscles. In such instances it is impossible to determine the limits of the ultimate branches of the fungus. Plate III, 3 and 4, are examples of the forms most commonly seen and these probably represent mature arbuscles. These degenerate after a time so that only the main branches are left in a collapsed condition (Plate IV, 3, 10 and 11). When this occurs the host tissue immediately surrounding is obviously dead and that section of the fungous thallus is empty of contents.

The mycelium also bears vesicles which are terminal enlargements of mycelial branches, more or less oval in shape (Plate III, 6) but tending to be somewhat distorted in the intercellular spaces. Only two examples (Plate IV, 12 and 13) of these structures were found in an intracellular position. In the living condition (Plate IV, 14 and 15) they closely resemble sporangia of the Phycomycetous type. Many of these were removed from the tissues and observed under conditions calculated to favor zoospore discharge, but they neither produced spores nor did they germinate by hyphae. The contents is oily in nature and when treated in lacto-phenol the small globules coalesce into one (Plate III, 6). It has been suggested that the vesicles may be storage organs.

Both arbuscles and vesicles may be produced simultaneously, or one or the other may be lacking. Vesicles may reach diameters of well over 100 microns, and they are very prominent in lacto-phenol preparations. They may be produced in such numbers as to cause visible distortion of the rootlet, the host cells being forced apart by the intercellular growth of masses of the fungus.

The designation of this organism as a Phycomycete by previous workers seems justified in view of its non-septate mycelium and the resemblance of the vesicles to sporangia, but it has no obvious relationship with other members of this group. No sexual process is known and with a single exception (Peyronel, 1923) no spores have been reported. There is little variation in the description of specimens of this fungus found in any of its hosts, and

failure to culture the organism has made it impossible to obtain direct evidence as to its specificity. Several weed hosts of this fungus may be found in close proximity to strawberry plants at Vineland and it seems unlikely that there would be strains specific to each.

The roots of young runners commonly become infected by mycelial strands from already infested roots nearby. Those which strike into the ground closest to the mother plant are often noticeably more heavily infected than those further away. In multiple infections of a single rootlet it has been observed that mycelium has travelled along the outside of the root, from a point where infection was established, to another part of the root. It seems likely that during the growing season most of the new infections come from living hosts. However, vesicles have been kept in dead host tissue and also free from host tissue in aqueous solutions for weeks without any disintegration of their contents or change in their appearance. Also, some infection has been produced in pots of Vineland soil in which there was probably no living host tissue to harbor the organism. Consequently it seems reasonable to suspect that the vesicles may function as resting bodies capable of maintaining the fungus in the absence of living hosts.

The fungus was active throughout the growing seasons of 1932 and 1933 at Vineland, in both wet and dry soils. It has been active in periods of mild weather in potted material left out of doors up to December 20, though the plants were dormant and the soil had been frozen solidly on several occasions. Jones' work with the similar fungus in legume roots showed that it was active at temperatures ranging from 9° to 33° C.

There is some question about the nature of the relation of this to very similar fungi in the roots of certain plants. Rayner (19) has discussed it in mutually beneficial relationships with several hosts. Jones (11), after a study of this fungus in legume roots, concludes that it is a parasite. O'Brien and M'Naughton (14) are of the opinion that this fungus is the primary cause of strawberry losses in Great Britain.

The evidence that this fungus is strictly a parasite when growing in the strawberry is indisputable. It has been noted that large amounts of cortical root tissues are killed after invasion. The activity of the fungus during a dormant period of the plant appears to preclude any mutually beneficial relation during that time; and, finally, clean-rooted strawberry plants have been grown through a complete cycle of dormancy, flowering and fruiting in steam-sterilized soil, so that we may discard any theory that the fungus is necessary to the plant. The writer considers that this fungus is one of the more important pathogenes of the strawberry at Vineland.

The Flora of Wild Strawberry Roots

Wild strawberries were collected in several parts of Ontario and from many habitats. The visible root flora parallels to a remarkable degree that found in cultivated varieties. The Phycomycetous mycorrhizal fungus, *Asterocystis* and *Rhizoctonia* are commonly present. The root symptoms as a whole resemble those of diseased cultivated strawberries. The fact that wild

varieties are affected with the same root rot that we have been dealing with is quite significant, for it is strong evidence that many of the strawberry parasites are indigenous.

Discussion

A slightly different method of attack has thrown new light on the problem of reduced yields of strawberries. Direct examination of roots showed that numerous fungi were associated with a root rot affecting practically every strawberry plant at the Experiment Station at Vineland. A study of these fungi has revealed the presence of primary parasitic forms belonging to four genera of the Phycomycetes and five genera of the Fungi Imperfecti, as well as some additional parasites which were occasionally seen but have not yet been studied in any detail.

The relative pathogenicity of these parasites is a problem of first importance under such conditions and yet it cannot be solved readily because the dominant parasitic flora varies from time to time. In some instances, as in the case of *Pythium*, moisture relations appear to control the seasonal activity of the parasite, in others temperature seems most important, but in many other cases it is impossible to evaluate the importance of the factor of competition and of the various physical and chemical factors of the soil environment. The fungi seen most commonly in microscopical examination of infected roots were the Phycomycetous mycorrhizal fungus, *Rhizoctonia* and *Asterocystis*, whereas the parasitic forms most commonly isolated in culture were *Pythium*, *Fusarium* and *Ramularia*. It is quite possible, however, that relatively short periods of activity of virulent parasites may be of more significance than the continued activity of less aggressive forms. It would seem, therefore, that a satisfactory answer to the question of the relative importance of these parasites could be obtained only by observation of their activities through several seasons.

It has been found very difficult to compare the conditions at Vineland with those described in the literature on strawberry root diseases. Several diseases of this type have been described and there has been a tendency to select a specific organism as the cause in most instances. Thus Alcock *et al.* attribute a root rot to a *Phytophthora;* O'Brien and M'Naughton, on the other hand, regarded the Phycomycetous mycorrhizal fungus as the fundamental cause of strawberry root rot in both Scotland and England. Walker (24) apparently regards species of *Ramularia* as the primary cause of "black root" in Ontario. This would seem to suggest fluctuations in the dominant parasitic flora at different times and places. In any case, our present knowledge of the strawberry root troubles at Vineland does not warrant the selection of any one organism as the primary cause.

Wardlaw (25) found a *Pythium* associated with a root rot in Scotland but considered that many fungi might be responsible for the disease. He noted that the trouble was more severe in poorly drained soils and advocated change in cultural methods. This suggests the possibility that the strawberries attacked may be of subnormal vigor. This is an interesting possibility which needs full investigation. The soil at Vineland was probably not seriously

deficient in nutrients and it was good in other respects. Yet, inasmuch as all the evidence suggests that the fungi involved are indigenous, there is the strong suggestion that cultural methods have somehow or other favored the accumulation of these parasites. The possibility of modifying the parasitic flora of the soil, of increasing it by continued cultivation of known forms and of decreasing it through cultural methods designed to increase the factor of competition with innocuous forms, must be investigated forthwith.

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RECENT OBSERVATIONS ON STRAWBERRY ROOT ROT IN THE NIAGARA PENINSULA[1]

BY A. A. HILDEBRAND[2]

Abstract

The investigations reported here have centred largely around isolations from, and critical microscopic examination of, the roots of affected plants. Isolations from 684 young, adventitious and lateral roots showing definite lesions bordered by turgid, healthy tissue, yielded representatives of approximately 20 different genera of fungi, ranging in frequency of occurrence from 0.4% in the case of *Gliocladium* to 32.7% in the case of *Fusarium*. Next to *Fusarium*, isolates of *Ramularia* and *Pythium* appeared most frequently and consistently in culture, the former in 28.5%, and the latter in 10.8%, of the plantings. Results of infection experiments involving these as well as other genera, including *Penicillium*, *Rhizoctonia*, *Hainesia*, *Cylindrocladium*, *Coniothyrium* and *Helminthosporium*, are not yet available and it is impossible at the moment to evaluate the significance of their association with the diseased condition of the roots from which they were isolated. Isolations from 125 apparently healthy rootlets occurring on root systems, other parts of which were more or less severely diseased, yielded representatives of nine different genera of fungi, ranging in frequency of occurrence from 1.6% in the case of *Alternaria* to 5.6% in the case of *Ramularia*. Isolates of *Fusarium* appeared in 4.0% of the plantings.

Microscopic examination of 550 adventitious and lateral roots of wild and cultivated plants has revealed the almost universal occurrence in their tissues of two of the so-called "endotrophic mycorrhizal fungi." One of these is of the characteristic phycomycetoid type, producing arbuscules and vesicles, the other, of the *Rhizoctonia* type familiar in orchids. The former type has been observed much more consistently than the latter. In a number of instances both types have been found in the tissues of the same rootlet. At least three strains of the *Rhizoctonia* type have been isolated but so far all efforts to culture the other organism have been unsuccessful. Resting spores of *Olpidiaster* (*Asterocystis*) and the spherical, smooth-walled spores of some member of the *Plasmodiophoraceae* were observed in diseased rootlets in the early part of the growing season. *Pythium* was noted in abundance occurring on diseased roots obtained in the field during September and November.

Nematodes have been encountered in association with diseased roots in numbers and frequency sufficient to suggest a possible causal relationship to strawberry root rot.

Introduction

Studies of root troubles of strawberries have of necessity become problems of major importance in many districts where this crop is grown under commercial conditions. Contributions in general but more particularly, perhaps, those in the field of pathological research have emphasized more and more the complexities of the problems involved. The results reported in the present paper, based upon investigations carried out during the past eight months, suggest that in the study of strawberry root rot as it exists in the Niagara peninsula the problems involved are no less complex than those encountered by workers elsewhere (Europe, U.S.A., etc.).

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Contribution No. 401 from the Division of Botany, Experimental Farms Branch, Department of Agriculture, Ottawa, Canada, forming part of a co-operative project between the Department of Plant Pathology, of the University of Toronto, and the Dominion Plant Pathological Laboratory at St. Catharines, Ont.

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Symptomatological Observations

In its general effect, in its wide distribution and in its more detailed symptomatology the disease referred to in this present paper as strawberry root rot compares more closely with the black-root disease of strawberries in Michigan as described by Strong and Strong (9) in 1931, than with any other root trouble described in the literature. In its general effect the disease is responsible not only for the failure of runner plants to become established in the soil, which characteristic effect was noted by Berkeley (2) as early as 1924, but also for sudden and heavy losses of older plants, particularly at the cropping period. With regard to more detailed symptomatology, the following observations may add certain points of interest to the symptomatological picture of the same disease as presented by Walker (10) in 1929. If during periods of thaw in the winter, daughter plants in their first period of dormancy are removed from the soil of plantations where the disease has been severe the previous summer, it will be found in many cases that the white, turgid, unbranched, autumn-formed adventitious roots are already more or less completely girdled by numerous, definite lesions, the roots at the point of attack showing dark brown to blackish discoloration and definite necrosis of tissue. As these adventitious roots "constitute the frame-work on which is built up the extensive fibrous root system necessary to support the plant through the flowering and cropping period" (4), it can be readily understood how the young plant in its subsequent development will be adversely affected in direct proportion to the severity of attack on its adventitious roots. If year-old plants growing in infested soil are examined in the spring or early summer, during which periods there is rapid and extensive development of lateral roots from the main adventitious roots formed the previous autumn, it will be found that many of the finer laterals have rotted off while others, still attached, show definite lesions of varying size. It may be noted here that O'Brien and M'Naughton (5) emphasize that the most characteristic symptom of the so-called "Lanarkshire strawberry disease" in Great Britain is one spoken of as "root weakness," the latter term being descriptive of a condition in which primary roots of affected plants lack lateral branches and finer fibres. Poverty of absorbing roots on affected plants is, then, a point in common between "Lanarkshire strawberry disease" in Great Britain and strawberry root rot in the Niagara peninsula. There is nothing, however, in O'Brien and M'Naughton's description of symptoms that suggests the occurrence on the larger adventitious roots, of definite lesions which, girdling such roots near the point of their emergence from the crown, render useless their entire distal portion with its more or less extensive system of laterals. Alcock (1) in examining diseased strawberry plants forwarded to her from various parts of Scotland noted that "the roots, or some of them, showed a definite die-back from their tips, a marked absence of laterals and of 'fibrous' root formation, and, less commonly than the tip die-back, intercalary lesions, *i.e.*, discolored areas on the surface tissues of the root not involving the tip."

The discoloration and collapse of tissue in diseased areas both on the autumn-formed adventitious roots and the spring-formed laterals are quite distinct from (i) the somewhat similar phenomena which accompany the seasonal cessation of function and death of the finer absorbing laterals, and (ii) the color changes and decortication of the older main roots which continue to live and become important regions of starch deposition. Symptoms attendant upon a diseased and deficient absorbing system are a general lack of vitality, a more or less stunted appearance, a reduced capacity for runner production and a sudden collapse at the onset of dry weather, especially during the cropping period. The increasing severity of the disease in a given plantation in successive years, the failure of second plantings on land where the disease has formerly been present and the gradual but certain spread of the disease throughout a given district, all point to the spread of inoculum and its accumulation in the soil to the point where it becomes "sick" for strawberries. The occurrence of lesions of such definite nature suggests attack by one or more parasitic organisms.

Isolations from, and Microscopic Examination of, Roots of Affected Plants

The work to date has involved isolations from, and detailed microscopic examination of, the roots of a large number of affected plants. The plants were obtained (i) from various plantations in the district at successive intervals throughout a period ranging from May, 1933, until January, 1934, and (ii) from greenhouse specimens obtained by training runners from healthy parents over pots containing soil collected at intervals throughout the growing season from plantations where the disease was severe. To avoid senescent tissue, material was carefully selected to include more recently formed and still-functional lateral and adventitious roots. Crowns were also examined and some were found to be diseased, but in the majority of such cases infection appeared to have spread to the crown from the roots.

A. Isolations from the Roots of Affected Plants

Individual roots or rootlets showing definite lesions were placed on a glass slide and washed under running water using a soft camel's-hair brush, the rootlet being held in position during the process by means of a rubber band passing around the slide and over one end of the specimen. Following rinsing in sterile water a small portion of the rootlet, including a necrotic region, was transferred to the surface of nutrient media in Petri dishes. In the case of almost every root system examined, individual rootlets were observed which, even under the dissecting microscope, showed no evidence of disease. A number of these apparently healthy roots, after being treated in identically the same manner as those showing definite lesions, were also transferred to plates of nutrient media. Surface sterilization of rootlets involving disinfectants was not attempted and non-acidified media were used throughout. Of various media tested earlier in the investigations, including prune agar, a malt-extract agar, strawberry root agar and potato-dextrose agar (dextrose, $2\frac{1}{2}\%$),

the last-mentioned proved to be most satisfactory and was used exclusively in the later work. Some of the plantings remained sterile, others produced bacteria alone, while the remainder developed both bacteria and fungi. As the fungi grew beyond the periphery of the bacterial colonies, hyphal tips were cut off and removed to tubed slants. Individual plantings yielded in some cases one or more representatives of a single genus, in others, representatives of several different genera. The complete results of the isolations from 684 diseased rootlets, that is, rootlets showing definite lesions, and from 125 apparently healthy rootlets of the same plants are summarized in Table I.

As will be noted in Table I, many different organisms have been found in association with a diseased condition of strawberry roots. Among the fungi, representatives of the genera *Fusarium* and *Ramularia* have been most frequently and consistently isolated, the former exceeding the latter in the aggregate by 4.2%. A close parallelism appears to exist in the occurrence of these two fungi. During June, July and September they were isolated with gradually increasing frequency until a peak was reached in November, after which, as shown by the results of January isolations, they fell off sharply. The various isolates of *Fusarium* have been resolved into five distinct strains or growth forms, while those of *Ramularia* have yielded at least six different forms. Strain differences in these and other genera encountered have been determined by comparison of morphological and cultural characteristics of monospore or hyphal-tip cultures grown for the same length of time on the same medium under identical environmental conditions. *Fusarium* and *Ramularia* appear to be of general distribution since they were isolated as frequently from affected plants from one plantation as from those from another. A parallelism is to be noted also in the occurrence of *Pythium* and *Rhizoctonia*. In July and August, during which periods the weather was unusually hot and dry, *Pythium* developed from only 3.5% and *Rhizoctonia* from only 4.4% of the plantings. In September, November and January isolations, the occurrence of *Pythium* increased to 15.8% (the average for the three months) and that of *Rhizoctonia* to 13.4%. Isolates of *Pythium*, which have yielded four different strains, have been obtained with equal frequency from plants from various sources, but the isolates of *Rhizoctonia*, which have also been resolved into four growth forms, have all been isolated from plants obtained from the same plantation where, during the summer of 1933, the disease was particularly severe. *Helminthosporium* (one strain) and *Cylindrocladium* (one strain), as in the case of *Rhizoctonia*, have been isolated from plants obtained in a particular plantation. Other fungi, however, including *Penicillium* (three strains), *Hainesia* (three strains) and *Coniothyrium* (one strain) appear to be general in their distribution.

Nematodes appeared frequently in the original plates but since, in many cases, their presence was not detected until the colonies of fungi or bacteria originating from different plantings had become confluent, it is impossible to tabulate the frequency of their occurrence with any degree of certainty.

Of a total of 125 plantings from what appeared to be healthy rootlets, 19 (15.0%) remained sterile, 64 (51.2%) developed bacteria alone and the

TABLE I

PERCENTAGE OCCURRENCE OF ORGANISMS ISOLATED FROM ROOTS SHOWING DEFINITE LESIONS AND FROM APPARENTLY HEALTHY ROOTS OF THE SAME PLANTS

Source of isolations	Roots showing definite lesions					Apparently healthy roots					Totals	
Time of isolations	June	July	Sept.	Nov.	Jan.	June	July	Sept.	Nov.	Jan.	Diseased	Healthy
Number of isolations	106	180	142	156	100	25	25	25	25	25	684	125
Bacteria alone	21.7	12.2	17.6	9.6	33.0	56.0	64.0	56.0	36.0	44.0	17.2	51.2
Fusarium	11.3	28.8	35.9	55.1	23.0	8.0	—	8.0	4.0	—	32.7	4.0
Ramularia	11.8	28.3	34.5	38.4	15.0	—	4.0	4.0	12.0	8.0	28.5	5.6
Plantings remaining sterile	24.5	—	—	—	—	8.0	12.0	16.0	20.0	20.0	3.8	15.0
Pythium	3.7	3.3	14.8	18.6	14.0	—	—	—	—	—	10.8	—
Unidentified fungi	19.4	12.1	1.4	10.2	7.0	—	4.0	—	8.0	4.0	9.6	3.2
Penicillium	—	13.8	9.8	1.2	13.0	—	4.0	4.0	—	—	7.8	1.6
Hormodendrum	—	13.3	4.2	—	—	12.0	4.0	4.0	—	12.0	4.3	6.4
Rhizoctonia	5.6	3.3	—	13.4	—	—	—	—	—	—	4.8	—
Aspergillus	—	—	2.1	—	2.0	4.0	—	—	4.0	4.0	0.7	2.4
Phoma	—	—	—	—	—	8.0	4.0	—	—	—	—	2.4
Cephalothecium	—	—	—	—	—	—	—	4.0	—	8.0	—	2.4
Rhizopus	1.8	—	—	8.3	2.0	—	—	—	—	—	2.4	—
Trichothecium	—	—	—	—	—	—	4.0	—	8.0	—	—	2.4
Alternaria	—	2.7	1.4	1.2	—	—	—	—	8.0	—	1.3	1.6
Hainesia	—	2.2	—	2.7	6.0	—	—	—	—	—	2.0	—
Cylindrocladium	—	—	7.0	—	—	—	—	—	—	—	1.6	—
Yeast-like fungi	—	—	—	1.2	1.0	4.0	—	4.0	—	—	0.4	1.6
Coniothyrium	1.8	1.1	—	—	2.0	—	—	—	—	—	0.8	—
Dematium	—	—	—	—	6.0	—	—	—	—	—	0.8	—
Torula	—	—	—	—	6.0	—	—	—	—	—	0.8	—
Helminthosporium	—	—	2.8	—	—	—	—	—	—	—	0.5	—
Verticillium	—	—	1.4	1.2	—	—	—	—	—	—	0.5	—
Actinomycetales	—	—	—	2.7	—	—	—	—	—	—	0.5	—
Gliocladium	—	—	—	0.7	2.0	—	—	—	—	—	0.4	—
Nematodes	Abundant					Rare						

remaining 42 (33.8%) produced bacteria and fungi. The development of bacteria from 85.0% of the plantings is not regarded as significant, since the rootlets previous to planting were washed in tap water only. Of the nine genera of fungi which appeared, representatives of six of them, namely, *Hormodendrum*, *Ramularia*, *Fusarium*, *Aspergillus*, *Alternaria* and *Penicillium* were also isolated from diseased rootlets. Whereas in plantings from diseased rootlets, representatives of several genera of fungi frequently developed from individual rootlets, healthy rootlets on the other hand in only a few cases yielded more than one genus. This is the reason why, in the section of Table I under "Totals," the percentage totals for isolations from diseased roots add up to 132.2%, while those for isolations from healthy roots total 99.8%. Nematodes were of rare occurrence in the five series of plantings from the healthy roots.

B. *Microscopic Examination of the Roots of Affected Plants*

In the microscopic studies, material from the roots of wild strawberry, *Fragaria virginiana*, has been examined in addition to the much greater amount of material obtained from roots of field and greenhouse specimens of several horticultural varieties of the cultivated strawberry. Material examined has included very young to older (but not senescent) adventitious, lateral and fibrous roots, some showing definite lesions, others exhibiting various degrees of discoloration and still others appearing perfectly healthy. The finer laterals *in toto* and razor sections of the larger and coarser adventitious roots were either (i) boiled for two to three minutes in lacto-phenol with acid fuchsin or cotton blue, or (ii) immersed in a solution prepared by adding 1 gm. of acid fuchsin to 100 cc. of picric acid (saturated solution in 95% alcohol). After boiling, the finer laterals were mounted in clear lacto-phenol and gently crushed under the cover slip. Altogether about 550 individual roots and rootlets from many different plants were examined in this way.

In the case of *isolations* it was possible to tabulate the results with a high degree of accuracy since the organisms having once been obtained in pure culture could be studied and identified at least as far as the genus in all but a comparatively few cases. Comparable accuracy is impossible in recording the results of microscopic examination. Too many cases were encountered where organisms that were present lacked the distinguishing characteristics necessary for even a generic diagnosis. Thus in the results which follow, only those organisms with reference to the identity of which little doubt remains have been mentioned, and no attempt is made to record their relative frequency of occurrence on a definite numerical basis.

In almost all roots, except those of plants grown in the greenhouse in autoclaved soil, the "endotrophic mycorrhizal fungus" of the phycomycetoid type as distinguished by Peyronel (6), bearing typical arbuscules and vesicles (Plate I, Figs. 1, 2 and 3), and already observed in the roots of a large number of species of plants of varied affinities by other workers and more particularly in the case of strawberry by Jones (3), Plakidas (7), O'Brien and M'Naughton (5) and probably by White (11, 12), has been found more frequently and

consistently than any other organism. It has never been observed deeper than the endodermis in the cortical tissues of very young adventitious roots, but it has been observed in great abundance in the same tissues of the finer absorbing laterals. Individual hyphal strands and small wefts of mycelium apparently loosely attached to the surface of roots but traceable to the internal tissues suggest the ability of the endophyte to develop outside of plant tissue. Vesicles and mycelium have been observed in equal abundance in specimens of four varieties collected from various plantations over a period ranging from May to November, but mycelium alone was noted in roots obtained in January. The profusely branched arbuscules (haustoria) showing dichotomy of the extremities of the finer branches have been observed much less frequently than the vesicles. The fungus occurs as commonly in rootlets which exhibit no macroscopic evidence of disease as in those which show discoloration and necrosis of tissue. No distortion such as reported by O'Brien and M'Naughton (5) has been noted in rootlets in which the endophyte was especially abundant (Plate I, Fig. 1). The fungus has not been observed in the stolons of plants with badly diseased roots.

Among the fungi, representatives of the genus *Rhizoctonia*, some ectotrophic, others endotrophic in habit, were noted second in frequency and consistency of occurrence. Endophytic forms produce in the root tissues (i) mycelial complexes which vary in size and resemble sclerotia, and (ii) monilioid chains resembling chains of conidia. Two forms which were obtained in the isolations from diseased roots, produce in addition, in culture, peculiar coils or skeins of mycelium. These two forms of the endophyte, morphologically very similar to one another, belong to the orchid type of mycorrhiza and resemble very closely (i) *Rhizoctonia repens*, which according to Rayner (8, p. 89) was isolated from a number of genera of orchids by Bernard in 1909, who illustrated the chains of conidia-like segments and coils of mycelium or "pelotons," and (ii) the form of *Rhizoctonia* which, also according to Rayner (8, p. 251) was isolated from wheat by Peyronel in 1924. Ectophytic forms of *Rhizoctonia* growing profusely over the surface of rootlets (Plate I, Fig. 7) were observed in many instances.

Resting spores of *Olpidiaster (Asterocystis)* (Plate I, Fig. 4) were quite commonly observed and the spherical, smooth walled spores of some member of the *Plasmodiophoraceae* (Plate I, Fig. 5) were occasionally noted in diseased rootlets of plants obtained in the field during May and June or grown in the greenhouse in soil collected in June. *Olpidiaster* was consistently more abundant in the more severely diseased rootlets. During the later summer and fall the fungus was noted in a few isolated instances only. Peyronel (6) reported that *Asterocystis radicis* was frequently encountered, both on healthy and on decayed roots of almost all the numerous plants he examined, and the organism appeared to be quite as constant an associate of these plants as phycomycetoid endophytes and those of the *Rhizoctonia* type.

Mycelium and spores of *Pythium* were noted in abundance in the region of lesions on rootlets of plants obtained in the field during September and early November.

In a single instance there was observed on the rootlet of a plant collected in the field in June a distinct acervulus, on conidiophores arising from which were borne two-celled spores, the distal cell of each spore being typically rostrate. In shape and size the spores correspond so closely to the conidia of *Diplocarpon earliana* described by Wolf (13) in 1926, as to suggest that this fungus, which up to the present has been reported only on the foliage and inflorescence of the strawberry, also occurs on the roots of that plant.

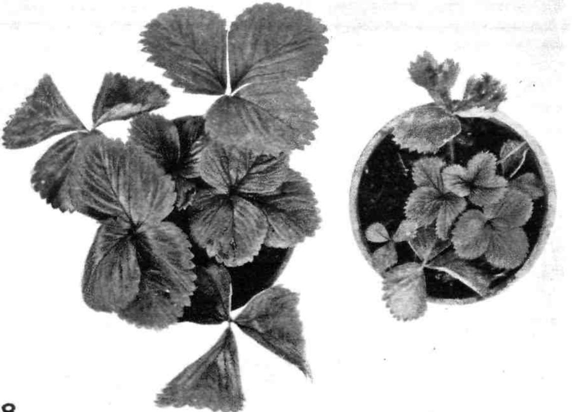
In one preparation, seven spores of *Ramularia* were observed scattered on the surface of a rootlet, this being the only definite, microscopic evidence of the presence of the fungus which, next to *Fusarium*, has been isolated most frequently from strawberry roots.

Nematodes have been observed in strawberry roots more frequently and consistently than any other organism, except the phycomycetoid endophyte (Plate I, Fig. 6). Whereas the endophyte has been found in many roots which showed no macroscopic evidence of disease, nematodes have been observed almost without exception in root tissues which showed definite discoloration and more or less necrosis of tissue. In such tissue and especially in what appeared to be incipient lesions, nematodes alone have been observed in many cases, the evidence suggesting that the diseased condition of the root could be attributed directly to their presence. Rootlets heavily infested with nematodes showed no malformations of the nature of knots or other enlargements. Nematodes have never been observed in the roots of plants grown in the greenhouse in autoclaved soil, but they have been noted occasionally in small numbers on what appeared to be healthy roots of field and greenhouse specimens, the latter grown in soil obtained from plantations where root rot had been severe.

Greenhouse Experiments, 1933

The occurrence of lesions on young and otherwise healthy adventitious and fibrous roots and the presence on the larger surviving laterals of large numbers of rotted "stubs" attesting the destruction of finer laterals after their formation (the disease first manifesting itself in the roots of plants), together with the evidence adduced from cytological studies of, and isolations from, roots of affected plants, strongly suggest that the disease is caused by one or more soil organisms. Additional evidence was sought in a series of preliminary experiments carried out in the greenhouse.

In May, 1933, 50 apparently healthy plants, variety Glen Mary, growing in the outside plots at the laboratory, were transferred to 9-in. pots in the greenhouse. Runners from these plants were allowed to strike in autoclaved compost soil in 7-in. pots. After the runners had become established they were detached from the parent plants and kept under observation. Without exception, healthy vigorous plants developed. On a microscopic examination of their roots no organisms could be found and when rootlets, following careful washing in sterile water, were transferred to plates of nutrient medium, the plantings yielded no growth of organisms, except bacteria in a few instances.



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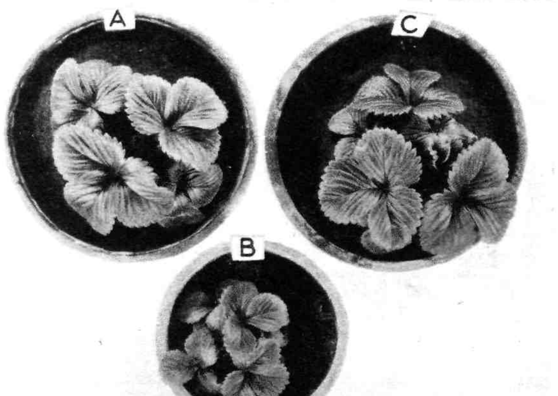

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FIG. 8. *Eighty-three-day-old, genetically identical runner plants, variety Glen Mary, the one (left) grown in autoclaved soil, the other (right) grown in soil obtained from a plantation where the disease was severe* ($\times \frac{1}{3}$). FIG. 9. *A, Fifty-three-day-old runner plant from healthy parent, variety Glen Mary, grown in autoclaved soil. B, Runner plant from parent A, grown for 44 days in soil obtained from a plantation where the disease was severe. C, Runner plant from parent B, grown for 30 days in autoclaved soil* ($\times \frac{1}{3}$).

Towards the end of June the daughter plants were in turn producing runners. On June 27, 25 of the most vigorous plants, each producing a pair of runners of approximately the same age, were selected. One runner from a given plant was trained over autoclaved compost soil in a 7-in. pot, while the other runner from the same parent was trained over soil obtained June 19 from a plantation where the most severe and typical case of root rot in the district had been encountered. Experiments exactly similar to the above but involving soils collected in July and September, were carried out later in the growing season. There was thus established in the greenhouse a population consisting of 75 pairs of plants, one component of each genetically identical pair growing in sterilized soil, the other in naturally infested soil.

The results obtained in all three series of experiments have been remarkably uniform. Almost without exception there has been a marked contrast between plants grown in sterile soil and those grown in naturally infested soil. The former have remained healthy, vigorous plants with well developed tops (Fig. 8, left) and root systems (Fig. 10, upper), and exhibit a remarkable capacity for blossom and stolon formation. The latter plants, on the other hand, have the appearance of "degenerate" plants. Their tops are stunted (Fig. 8, right) and their root systems are more or less diseased and deficient in the finer absorbing laterals (Fig. 10, lower). They exhibit very limited capacity for blossom and stolon formation. Their foliage, though dwarfed and at certain stages slightly lighter in color than that of plants grown in autoclaved soil, exhibits no other alteration in habit or symmetry. To date, six of the plants grown in the infested soil have died and it appears that a number of

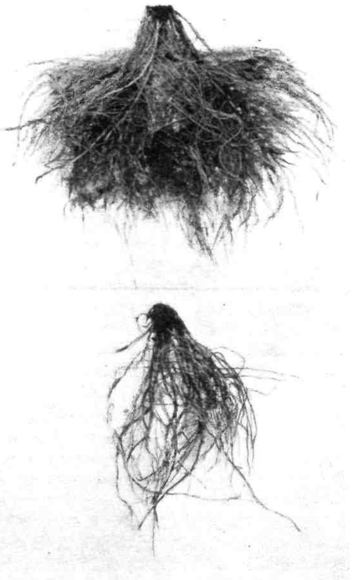

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FIG. 10. *Root systems of forty-four-day-old, genetically identical runner plants, variety Glen Mary, the one (upper) grown in autoclaved soil, the other (lower) grown in soil obtained from a plantation where the disease was severe.*

others cannot survive much longer. Nematodes and the varied fungous flora recorded earlier in the present paper were found in abundance, constantly associated with the diseased condition of the roots of these plants grown in the infested soil. Careful microscopic examination of the roots of plants grown in autoclaved soil has never revealed the presence of any organism, and plantings from them to nutrient media have yielded bacteria only. The latter plants have matured fruit without the "mycorrhizal endophyte" being present in their roots.

To determine whether the disease could be transmitted through the stolon to the runner progeny, the following experiment was carried out. Fifteen of the "degenerate" plants, which at various times throughout the growing season *did* produce stolons, were selected and the latter were trained over autoclaved compost soil. All 15 runners developed into vigorous, healthy plants (Fig. 9) in the roots of which it has so far been impossible to demonstrate the presence of any organism.

Discussion

Although the investigations to date have not progressed appreciably beyond the stages of a preliminary survey, the evidence which has already been adduced is regarded as being sufficient to warrant the view that fungi in particular, and possibly nematodes, are two of the more important members of a complex of factors responsible for strawberry root rot. Isolations from carefully selected material have yielded representatives of approximately 20 different genera of fungi, ranging in frequency of occurrence from 0.4% in the case of *Gliocladium* to 32.7% in the case of *Fusarium*. Representatives of the genera *Fusarium*, *Ramularia* and *Pythium* have been isolated most frequently and consistently from typically diseased roots but, as the results of infection experiments involving these and other genera are not yet available, it is impossible at the moment to evaluate the significance of their association with the diseased condition of the roots from which they were isolated. It may be pointed out that the fungi isolated include *Cylindrocladium* and *Helminthosporium*, representatives of which genera apparently have not heretofore been isolated from *Fragaria* spp.

It was noted in the laboratory when strain differences in several of the genera mentioned above were being investigated, that certain of the strains of *Ramularia* differed markedly in their temperature requirements, not only from one another but also from certain strains of other genera, particularly *Hainesia* and *Fusarium*. It was noted in the field that after a particularly heavy rainfall (1.19 in.) on June 8, 1933, followed by unusually high temperatures for several days (82.2° F. mean maximum temperature for three days), a severe outbreak of root rot occurred in a particular plantation where previously there had not been sufficient disease to attract attention. These observations and others of a confirmatory nature would suggest that of many possible factors involved in the occurrence of the disease, the two variables, temperature and moisture, are by no means the least important and that

in infection experiments, particularly, these two factors should receive careful consideration if reliable data as to pathogenicity are to be obtained.

Of interest in the present studies has been the discovery of the almost universal occurrence in the roots of strawberries, of two fungi, one, of the characteristic phycomycetoid type producing arbuscules and vesicles, the other, of the *Rhizoctonia* type familiar in orchids. The former type has been observed much more consistently and abundantly than the latter. In a number of instances both types have been found in the tissues of the same rootlet. At least three strains of the *Rhizoctonia* type have been isolated from diseased rootlets, but all efforts to culture the phycomycetoid type have been so far unsuccessful. According to Rayner (8, pp. 250–253), Peyronel in 1924 published a long list of species of plants including the strawberry, distributed among 37 families, in which he had found both types of organisms. His observations led him to conclude that "endotrophic infection" in a majority of mycorrhizal plants is of a composite character and that two distinct stages of infection may be recognized; an earlier phase depending upon invasion by the phycomycetoid type and a later phase in which an organism of the *Rhizoctonia* type is also present. He isolated the endophyte of the orchidaceous type from the roots of many plants including cereals, potato, tobacco and vine but he did not succeed in culturing the endophyte with arbuscules and vesicles. In addition to Peyronel other workers both in America and in Europe who have observed "endotrophic infection" include Jones (3), White (11, 12), O'Brien and M'Naughton (5) and Plakidas (7) all of whom, however, appear to have noted only the phycomycetoid type of endophyte. None of these workers, with the possible exception of White (12), have succeeded in obtaining the endophyte in pure culture.

With regard to the relation of the phycomycetoid type of endophyte to pathological conditions of the strawberry, O'Brien and M'Naughton (5), on the strength of historical, mycological and field evidence but without having cultured the organism, conclude not only that the endophyte is a definite parasite but also that it is the primary and underlying cause of the general disease known as "root weakness" affecting strawberries throughout Great Britain. They regard the so-called "Lanarkshire strawberry disease" as representing this trouble in its most serious form. In their opinion the endotrophic mycorrhiza paves the way for the entry of secondary fungi and bacteria, which under certain conditions, may invade the weakened root tissues and intensify the disease. Plakidas (7) in his investigations as to the cause of strawberry xanthosis examined the roots of a large number of plants, both healthy and diseased. In both he found almost invariably what appeared to be a mycorrhizal fungus identical with that which Jones (3) had observed in the roots of strawberry and many other plants. Plakidas notes that "invaded roots appear perfectly normal externally and no lesions occur in the tissues in which the fungus is found." He made no attempt to isolate the fungus since he was assured that it bore no causal relationship to xanthosis. At the present time it is impossible to express an opinion as to the relation

of "mycorrhiza" to pathological conditions in roots of the strawberry in the Niagara peninsula. However, the killing of cells of the cortical region together with the disappearance of the contents of other cells of the same region in roots heavily infected with the phycomycetoid type of endophyte, as revealed by microscopic examination, suggests a parasitic rather than a symbiotic relationship. The latter type of endophyte has been found (i) in the roots of wild strawberry growing in virgin soil, (ii) in the roots of the healthiest plants that could be found in certain few plantations where indications of the presence of root rot could not be found, and (iii) in the roots of plants obtained from plantations where the disease was present, the specimens examined including plants showing various stages of the disease. In brief, the fungus has been found almost invariably in all plants examined except those grown in autoclaved soil in the greenhouse. The latter appear to have benefited when protected from root infection by sterilization of the soil before planting. Even if the endophyte were responsible for the diseased condition and loss of the finer absorbing laterals, the occurrence of lesions on otherwise healthy and functional adventitious roots remains to be accounted for. The organism has been observed in the latter only in very early stages of their development.

The abundance and consistency of the occurrence of nematodes definitely within the tissues of diseased rootlets, and in many cases the apparent absence from such tissues of any other organism, render it imperative to regard them tentatively at least as bearing a possible important causal relationship to strawberry root rot.

In connection with the greenhouse experiments, several reasons may be advanced to account for the striking and consistent difference that exists between genetically identical plants grown, on the one hand, in autoclaved soil and, on the other, in soil obtained from plantations where the disease was severe. It has been known for a long time that sterilization of soil in some way results in its increased fertility. This might be one reason why plants grown in the autoclaved soil are more vigorous and healthy than those grown in the non-sterilized soil. But, in the case of the latter plants, while lack of soil fertility might be held accountable for limitations in their general development, with perhaps consequent increased predisposition to attack by parasitic organisms, it does not account for the actual occurrence of lesions on the rootlets of young runner plants and for the later more general diseased condition of their root systems. While the evidence strongly suggests that soil organisms are primarily responsible for the "degenerate" condition of these plants, it is not considered impossible that other agencies capable of inducing or contributing to the same general symptomatological picture may be involved.

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NOTE ON A NEW *OPHIOBOLUS* ON EELGRASS

Ophiobolus halimus Diehl and Mounce n. sp. is reported on *Zostera marina* L. from St. Andrew's, N.B. It was collected on rhizomes and fertile shoots, and developed in leaves kept in sea water in the laboratory. The fungus will be described and illustrated and will be compared with related species in the August issue of the Canadian Journal of Research.

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STUDIES ON THE DISTRIBUTION OF MOLYBDENUM IN BIOLOGICAL MATERIAL

I. A SPECTROGRAPHIC STUDY OF THE OCCURRENCE OF MOLYBDENUM IN PLANTS GROWN IN THE PROVINCE OF QUEBEC[1]

By Andrew Dingwall[2], R. R. McKibbin[3] and H. T. Beans[4]

Abstract

Molybdenum, identified spectrographically by the lines 3902.9Å, 3864.1Å, 3798.3Å, 3193.9Å, 3170.3Å, 3158.1Å and 3132.6Å, was found to be present in vetch, alfalfa, alsike, red clover, timothy, goldenrod, reed canary grass, field corn stalks, Canada thistle, ragweed, celery, beets and carrots grown on a farm in Jacques Cartier county, Quebec. Molybdenum could not be detected in the soil on which the plants grew.

Molybdenum was not found in vetch plants grown on several farms in Brome, Compton and Sherbrooke counties; nor in timothy, alsike clover or red clover grown on a farm in Brome county; nor in lettuce, celery, cabbage, beets and carrots grown on two different muck soils of the St. Lawrence River valley, in Huntingdon county.

The farm in Jacques Cartier county lies in the Ottawa River valley drainage basin, the other farms do not. Known deposits of molybdenum occur in the Ottawa River valley.

Introduction

Many attempts have been made to determine which elements are necessary for life, and as a result of the investigations it is generally agreed that certain elements must be present before normal growth can take place. Whether or not some other elements, among which is molybdenum, are essential, is somewhat uncertain. This lack of knowledge is due largely to the inadequacy of the methods available for their detection in very minute quantities. The present study deals with the occurrence of molybdenum in plants grown in a region of the Province of Quebec.

Historical

Molybdenum is not found free in nature. Molybdenite, MoS_2, was known to, and used by, the ancient Greeks but it was not until 1782 that Hjelm (9) isolated the metal. Molybdenum is relatively scarce in the earth's crust, being present, it is estimated, to the extent of about $10^{-7}\%$.

Jorissen (8) found molybdenum in the flue dust from burned Liège coal. Molybdenum was reported to be present in the ash of trees by Demarcay (5) who made an aqueous extract of the ash and examined spectrographically the precipitate obtained by means of hydrogen sulphide. Molybdenum was identified as "three strong lines in the ultra-violet in the neighborhood of K", an identification that leaves much to be desired. Bardet (1) reported molybdenum to be present in sea water, and Cornec (4) made a spectro-

[1] *Manuscript received April 19, 1934.*
Contribution from the Spectrographic Laboratories, Department of Chemistry, Columbia University, New York, U.S.A., and the Department of Chemistry, Macdonald College, Quebec, Canada.
[2] *Research Associate, Columbia University.*
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[4] *Professor of Chemistry, Columbia University.*

graphic study of the group precipitates obtained from aqueous and acid extracts of about three kgm. of ash from *Laminaria*. He reported molybdenum to be present but gave no details of the method of analysis. Steiner (14) reported the presence of molybdenum in a mineral water and determined its concentration as 0.8 mgm. molybdic oxide per litre, using a colorimetric method based on the reaction of molybdate ion with tannin. ter Meulen (10) published a very extensive piece of work on the occurrence of molybdenum in a wide variety of biological products including soils, plants, animal organs, mineral waters, coal ash and petroleum. In the case of the coal ash 64 kgm. was used to make the test and from this 251 mgm. of molybdic oxide was obtained. The quantitative determinations were based on a colorimetric method involving the color developed when sodium sulpho-molybdate is boiled with ammonium chloride. The author admits that the errors in the colorimetric method are relatively large, and in view of the fact that the final analysis is based on the product obtained by chemical separation of minute amounts of material in the presence of large amounts of other elements, it is more than probable that the quantities recorded are much lower than they ought to be. In a later paper (11) he reports the occurrence of molybdenum in dried algae, although he was unable to find molybdenum in 40 litres of sea water.

Ramage (13), using spectroscopic methods, reports molybdenum as present in the flue dust from S. Yorkshire coal in the Norwich Gas Works.

The role of molybdenum in soil biology has claimed some attention. Bortels (2) reported that the growth of *Azotobacter chroococcum* in a nitrogen-free medium was stimulated by the addition of sodium molybdate.

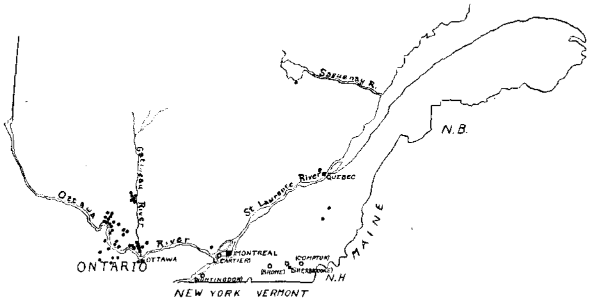
Forjaz (12, p. 829) claimed that the soil elements active in nitrogen fixation are molybdenum, nickel and cobalt. He based this deduction on the difference in the spectrographic analyses of the ashes from the roots and nodules of *Lupinum luteus*, the elements being present in the nodules but not in the roots. A critical examination of the data recorded leads to the conclusion that the elements were not properly identified. No details were given to show how the wave-lengths were measured, nor to indicate the accuracy of the measurements. The fact that the most sensitive spark lines of nickel were not found would indicate that the lines listed are not really nickel lines. The same is true of molybdenum. None of the recognized spark *raies ultimes* of molybdenum are listed, while a number of weak molybdenum spark lines are recorded.

Burch-Herschfeld (3), studying the influence of molybdenum and soil extract on the nitrogen assimilation of *Azotobacter chroococcum*, found that soil extract and sodium molybdate separately increase nitrogen fixation but in combination the effect is slightly less than that of either alone. The molybdenum does not increase the rate of vegetative growth on the soil. The ash of the soil extracts has the same effect as molybdenum. Goldschmidt and Peters (7) found molybdenum in the ash from coal, shale and peat from Germany and England.

Types and Sources of Samples Used in the Present Study

Source of the Samples

Seven specimens of wild blue vetch, one of common red clover, one of timothy, and one of alsike clover were collected in 1931 from farms in Brome, Compton and Sherbrooke counties.



Map of the southern part of the province of Quebec—Black circles indicate known molybdenum occurrences. Open circles show the general locations from which soil and plant samples were taken.

Wild blue vetch was collected from the 1931 and the 1932 crops grown on the muck and clay soils of a farm in Jacques Cartier county, just above the junction of the Ottawa and the St. Lawrence rivers. Specimens of alfalfa, common red clover, timothy and alsike clover were also collected. In 1932, reed canary grass, field corn stalks, Canada thistle, goldenrod and ragweed, and in 1933, celery, beets and carrots were obtained from the muck soil.

From Huntingdon county, on the St. Lawrence River, about 30 miles west of the Ottawa-St. Lawrence confluence, carrots, beets, lettuce, celery and cabbage were obtained from two muck soils in 1933.

Five specimens of soil, surface soil and subsoil, were obtained from the sources shown in Table I.

TABLE I

SOURCES AND TYPES OF SOILS STUDIED

Type of soil	Source
Appalachian upland region	
Virgin upland podsol	Foster, Brome county, Quebec
Virgin upland podsol	Mansonville, Brome county, Quebec
Virgin brown forest	Sutton, Brome county, Quebec
Virgin brown forest	Knowlton, Brome county, Quebec
Ottawa River valley	
Virgin lowland muck swamp	St. Anne de Bellevue, Jacques Cartier county, Quebec

In all cases the sample taken included more than one plant; never fewer than six plants, and in most cases, 12 or more were used. Only the roots of the beets and carrots, and only the tops (stems, leaves and floral parts) of the other plants, were tested. The soils examined were those in which plants collected were actually growing. Approximately five-pound samples were collected from surface soils to a depth of 8 in., and from subsoils between depths of 16 and 24 in.

Analytical Procedures

Preparing the Samples

When collected in the field the plants were placed in paper bags and brought to the laboratory. The samples of vetch, alfalfa, alsike, red clover, timothy, goldenrod, reed canary grass, field corn stalks, Canada thistle and ragweed were then dried in a fume-free atmosphere. These dried plants were cut up in a Wiley laboratory mill until they would pass a screen with 0.5 mm. meshes, prior to ashing. All the beets, cabbage, carrots, celery and lettuce plants sampled were, while fresh, cut up with stainless steel knives, placed in platinum dishes and dried to constant weight in an electric oven, prior to ashing. In the laboratory the soil samples were air dried and then pulverized, using an iron mortar and pestle.

Ashing Equipment

Fig. 1 shows the equipment used for ashing the specimens, together with the apparatus employed in the purification of the graphite electrodes. The glassware is of Pyrex.

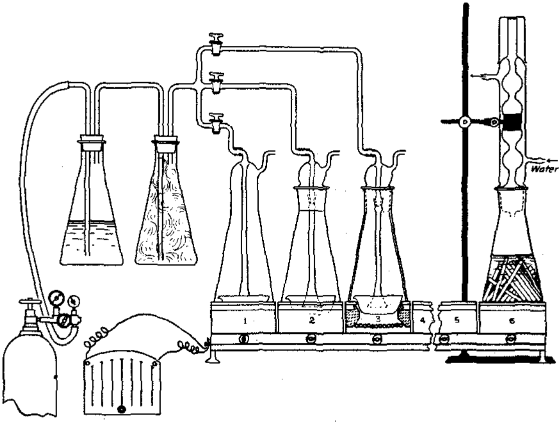


FIG. 1. *Ashing and graphite purification equipment.*

Two types of ashing equipment are shown. No. 1 is made of one piece, while Nos. 2 and 3 are made of several pieces connected by means of standard ground glass joints. Both types consist essentially of a glass hood which rests on a ground Pyrex glass plate in which is a hole cut to the size of the platinum basin. A funnel is sealed into the top of the hood to permit an atmosphere of oxygen to be maintained during the ashing process. A variable resistance is placed in series with the nichrome heating unit, while the last three heaters are connected in parallel through the same resistance.

Ashing the Specimen

Approximately 10 gm. of the specimen was weighed into a clean platinum basin which was placed in the hole in the Pyrex plate. The glass hood was separated from the plate by a fine platinum wire to prevent the cementing of the hood to the plate by the tarry distillate during the early part of the ashing. The resistance was so adjusted that the platinum basin never showed the faintest trace of red during ashing. The oxygen was bubbled through distilled water, then passed through a tower of absorbent cotton. When the destructive distillation had ceased the basin was allowed to cool, the mass broken up with an agate pestle and the ashing was then continued until the ash was white. No attempt was made to continue the ashing until constant weight was obtained.

Purifying the Graphite

Acheson $\frac{3}{8}$ in. spectroscopic graphite was cut into appropriate lengths (approximately 3 in.) and the face machined. They were arced for a few minutes to render the surface more porous, then refluxed with sulphuric acid (1–1) for 24–30 hr. Part of the acid was replaced with water and finally the graphite was refluxed with many portions of distilled water. The graphite, before use, was placed on a platinum triangle and heated to bright redness to drive off any traces of water, etc., that may have been left.

Graphite thus prepared shows negligible traces of sodium, magnesium, silicon and boron only, when arced for 10 min. at 10 amp., using a condensing lens.

Preparation of Silver Electrodes

Silver rods of high purity, $\frac{3}{8}$ in. in diameter, were obtained from Messrs. Handy and Harmon, Bridgeport, Conn. The rods were cut into pieces usually 3 in. long aud the faces machined. These pieces were then heated in concentrated hydrochloric acid, well washed with water, the tips only immersed momentarily in hot nitric acid (1–1) and well washed with distilled water. The two last-mentioned operations were repeated several times. The water was then shaken off the surface and the metal mounted in the electrode holders.

Arc Stand

Fig. 2 shows the arc stand used. The base, of cast iron, is bolted to the head of the machine lathe slide rest and levelled by means of the set screw *A*.

The uprights and the left- and right-handed screw and nuts are of Monel metal; the electrode holders are of invar. The insulation material is bakelite and transite lumber, and the bushings are of phosphor bronze. The driving tube is mounted on ball bearings. The combination pulley and radiator is of duralumin.

Horizontal adjustments may be made in two directions and the vertical adjustment is carried out by loosening the thumb screws *B* and lifting to the required height when the screws are again tightened.

The electrodes are clamped in the holders by the screws *C* and the electrodes are automatically centered by having each of them project an equal distance from the holder.

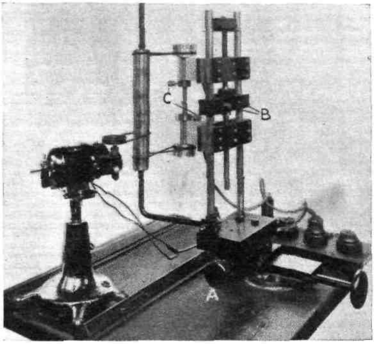


FIG. 2. *The arc stand.*

Making the Spectrograms

The electrodes having been satisfactorily lined up with respect to the spectrograph, a control exposure of the electrodes was made using the top opening in the Hartmann diaphragm. The arc was operated at 220 volts d.c., using 4 amp., the length of the arc being 2 mm., a 10 min. exposure of the photographic plate, Eastman 33, being given. The arc having been extinguished, 5 mgm. of the ash to be examined was weighed into the platinum weighing scoop. The ash was transferred to the lower, positive, pole and the arc again struck with another electrode held in an ordinary spring clothes pin. A 10 min. exposure was given through the middle opening of the Hartmann diaphragm. Fresh electrodes were placed in the holders, the diaphragm was moved over until the slit was exposed through the bottom opening, a trace of ammonium molybdate was added to the lower electrode, and the arc was struck. The plate was developed, using Eastman developer D28, fixed, washed and dried.

Silver electrodes were used for the region 3250Å to 8600Å, while graphite electrodes were used in the region 2450Å to 3300Å.

Identification of Molybdenum

A line in a spectrum is identified by its wave-length. The precision necessary if such a measurement is to be significant is usually not attainable with the type of spectrograph used for analytical purposes. The method commonly employed is to make spectrograms of the unknown and of the element

being sought by some method similar to that just described. If the element sought is present in the unknown then there will be, depending on the quantity present, a line or series of lines common to both spectrograms. It does not necessarily follow, because such a state of affairs is found, that the identification can be considered complete. Coincidences due to the presence of elements with complex spectra, *e.g.*, iron, etc., may be expected. There are a large number of lines in the molybdenum spectrum but as the quantity of molybdenum used is decreased fewer lines are seen, until when only a very minute quantity is present, only the following lines remain,—3902.96Å, 3864.12Å and 3798.26Å. These are the lines first sought. But the specimens contain an appreciable amount of iron and two iron lines are found,—3902.95Å and 3798.51Å. With the type of spectrograph employed in the present investigation, a Hilger E1, the dispersion in this region is not sufficiently great to enable the molybdenum lines 3902.96Å and 3798.26Å to be differentiated from the iron lines under certain circumstances. When the iron content is low and the molybdenum content relatively high there is no necessity for such differentiation, but where the iron content is high and the molybdenum low these lines cannot be used. Identification then depends on the line 3864.12Å.

Where only traces of molybdenum are present the problem is still further complicated by the fact that the so-called cyanogen bands are very pronounced in this spectral region. If graphite electrodes are employed relatively short exposures must be given to reduce the band structure to a minimum, and this results in decreased sensitivity. On the other hand, metallic electrodes can be used but again the cyanogen bands are found, owing to the carbon dioxide and nitrogen of the air, although very much reduced in intensity when compared with graphite. There are other molybdenum lines that are quite sensitive, 3193.98Å, 3170.35Å, 3158.16Å and 3132.60Å, and in a region free from carbon bands, but again the identification is complicated by the presence of band structure when traces only are being sought.

In the samples grown in Jacques Cartier county both sets of lines were found so that no difficulty was experienced in identifying molybdenum, but in the case of the other specimens failure to find the line 3864.12Å, using silver electrodes, was taken as an indication of the absence of molybdenum within the limits of the sensitivity of the test.

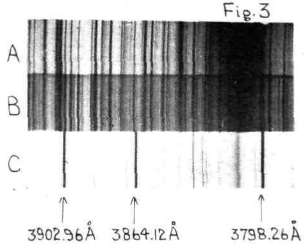
Results

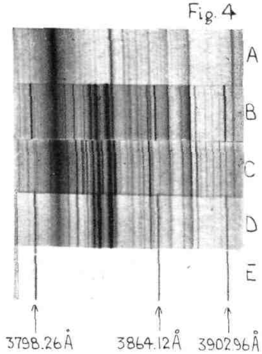
The following plants grown on a farm in Jacques Cartier county were found to contain molybdenum: Vetch (crop of 1930 and 1931), field corn stalks, red clover, Canada thistle, timothy, ragweed, alfalfa, celery, alsike, beets, goldenrod, carrots, reed canary grass and vetch from clay soil.

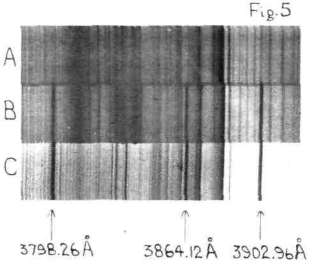
Molybdenum was not found in any of tbe other plant specimens examined, nor was it found in any of the soils.

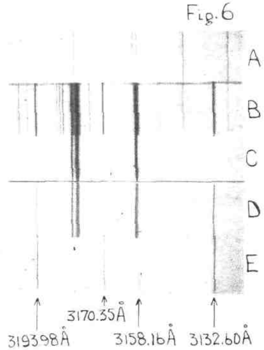
Discussion

Although molybdenum could not be demonstrated in either the subsoil or the surface soil of the Jacques Cartier county muck, yet a wide variety of









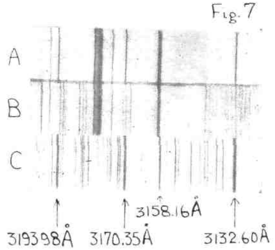


FIG. 3. *A and B, spectrogram of ash of soil from Jacques Cartier county, Que.—A, muck subsoil; B, muck surface soil; C, spectrogram of molybdenum.* FIG. 4. *A, spectrogram of silver; B, C and D, spectrograms of ash of vetch grown in—(B), muck soil, Jacques Cartier county, Que.; (C), Brome county, Que.; (D), Clay soil, Jacques Cartier county, Que.; (E), spectrogram of molybdenum.* FIG. 5. *A and B, spectrograms of ash of red clover grown in—(A), Brome county, Que.; (B), muck soil, Jacques Cartier county, Que.; (C), spectrogram of molybdenum.* FIG. 6. *A, spectrogram of graphite. B, C and D, spectrograms of ash of vetch grown in—(B), muck soil, Jacques Cartier county, Que.; (C), Brome county, Que.; (D), clay soil, Jacques Cartier county, Que.; (E), spectrogram of molybdenum.* FIG. 7. *A and B, spectrograms of timothy grown in—(A), muck soil, Jacques Cartier county, Que.; (B), Brome county, Que.; (C), spectrogram of molybdenum.*

plants grown on that soil contained appreciable quantities of molybdenum. The molybdenum must have been present in the soil solution and the question naturally arises as to where, and what, was the origin of the molybdenum.

The molybdenum may have been present in the rock originally occupying the site or it may have been carried there by water from a source at a higher altitude. The latter is the more probable explanation as the farm in question is in the Ottawa River valley and the Ottawa River drains a large area in which molybdenum was mined during the Great War. In Quebec this region lies in the main between the towns of Waltham on the west and north and Buckingham on the east and south, with several known deposits somewhat north of Maniwaki. In Ontario the known deposits lie between Pembroke to the west and north and Smith's Falls to the east and south. This general zone of molybdenum occurrence in pre-Cambrian igneous or metamorphosed rocks is bounded roughly on the west in Ontario by the Muskoka lakes district and in the east in Quebec by the valley of the Gatineau River, a tributary of the Ottawa River (6).

There are two reported occurrences of molybdenum in Megantic county, Quebec, in amorphous rocks of the Appalachian mountain region 50 miles north of the area in which the samples from the Appalachian region reported in this article were taken. The drainage is away from the region in which the specimens were collected.

The results of the investigations on the occurrence of molybdenum in animals will appear elsewhere shortly.

Acknowledgments

The spectrographic laboratories of the Department of Chemistry of Columbia University were founded by the Royal Baking Powder Co., and have received generous aid from Dr. and Mrs. Reginald Auchincloss. The authors are indebted to Professor J. F. Snell, Chemistry Department, and Acting Dean of Agriculture, Macdonald College and to Professor H. R. Murray, Horticulture Department, for their co-operation in obtaining and providing some of the specimens for analysis.

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ADDITION REACTIONS OF VINYL PHENYL KETONE

IV. TRIMOLECULAR PRODUCTS[1]

By C. F. H. Allen[2] and A. C. Bell[3]

Abstract

Methyl cyanoacetate, cyanoacetamide, and malonitrile readily add to vinyl phenyl ketone to give trimolecular products, while nitromethane gives a substance composed of one molecule of nitro compound and three molecules of unsaturated ketone. The reactions of the delta ketonic nitrile formed from the ester are described in detail; it is transformed into reduced pyridine derivatives, and a secondary open chain nitrile. The latter, in turn, is converted into pyridine and hydropyridine derivatives.

In the earlier papers in this series, the bimolecular addition products formed by the addition of phenylnitromethane (2), desoxybenzoin (1), and methyl malonate (3) to vinyl phenyl ketone were described. The first trimolecular product was encountered with methyl malonate, but it did not interfere with the main problem. In many cases, however, the only products that can be obtained are those composed of two molecules of ketone and one of addend. This would indicate that the primary addition product is more active than the original addend; in confirmation, even when the latter is used as a solvent, the substance found is the trimolecular product; *e.g.*,

$$\begin{array}{c} CH_2{=}CHCOC_6H_5 \\ \\ + CH_2(CN)COOCH_3 \end{array} \longrightarrow \begin{array}{l} CH_2CH_2COC_6H_5 \\ | \\ CH(CN)COOCH_3 \\ + CH_2{=}CHCOC_6H_5 \end{array} \longrightarrow \begin{array}{c} CH_2CH_2COC_6H_5 \\ | \\ NC{-}C{-}COOCH_3 \\ | \\ CH_2CH_2COC_6H_5 \\ (I) \end{array}$$

In the homologous series starting with benzalacetophenone, the same phenomenon is encountered, but to a less extent, since the primary addition product can be isolated in good yields in almost every instance. The difference, doubtless, may be attributed to the effect of the phenyl group on the beta carbon in this series.

In this paper will be described the products resulting from the addition of methyl cyanoacetate, cyanoacetamide, malonitrile, and nitromethane to vinyl phenyl ketone.

The methyl cyanoacetate addition product (I) is a delta ketonic nitrile, and differs from those (II) previously described in the location of the substituent groups, having only hydrogen atoms on the beta carbon atom, and no hydrogen

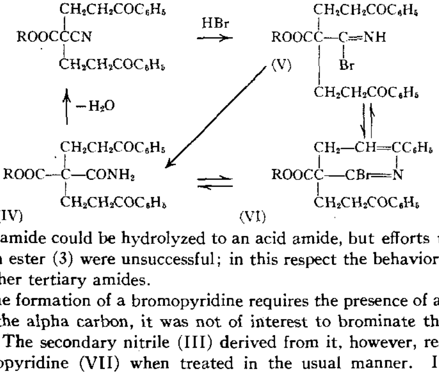
$$\begin{array}{l} RCHCH_2COR \\ | \\ RCHCN \\ \\ \quad (II) \end{array} \qquad \qquad \begin{array}{l} CH_2CH_2COC_6H_5 \\ | \\ CHCN \\ | \\ CH_2CH_2COC_6H_5 \\ \qquad (III) \end{array}$$

atom on the alpha carbon.

[1] *Manuscript received May 11, 1934.*
Contribution from the Chemical Laboratory of McGill University, Montreal, Canada.
[2] *Assistant Professor of Chemistry, McGill University.*
[3] *Graduate Student, McGill University.*

The two characteristic reactions of delta ketonic nitriles are—(*a*) the formation of tetrahydropyridine derivatives by the action of mineral acids, and (*b*) the production of 2-halopyridines when treated with halogens. Various intermediate products are known in both series and the mechanism is clear.

The ester-nitrile (I) is converted to an ester-amide (IV) when treated with hydrogen bromide in the usual manner. When the operation is conducted in chloroform solution, the substance which separates has the properties of an "imide bromide" (V); it is very easily hydrolyzed to the amide (IV). The latter is the end-product if acetic acid is employed as a solvent, but in this case, by interrupting the process, a third substance can be isolated. Analysis indicates that the latter is a dehydration product of the "imide bromide"; it contains one double bond, and can be converted easily to the amide or "imide bromide". For these reasons, the substance is best represented as the dihydropyridine (VI). There is no evidence that either of these substances is an intermediate in the formation of the amide, but the relation between them can be indicated as follows:



(IV) (VI)

The ester-amide could be hydrolyzed to an acid amide, but efforts to prepare the known ester (3) were unsuccessful; in this respect the behavior resembles that of other tertiary amides.

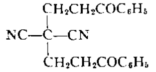
Since the formation of a bromopyridine requires the presence of a hydrogen atom on the alpha carbon, it was not of interest to brominate the addition product. The secondary nitrile (III) derived from it, however, readily gave a 2-bromopyridine (VII) when treated in the usual manner. It was impossible to stop the reaction until two molecules of bromine had been used up, one of which entered the side chain. Both atoms of bromine are removed by sodium alcoholates, but potassium acetate takes out only one, presumably alpha to the carbonyl group, since other 2-bromopyridines are unaffected by this reagent.

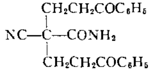
(VII) (VIII)

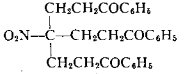
The replacement of R (Formula II) by hydrogen, therefore, does not make any difference in the result of bromination.

The secondary nitrile was isomerized to the tetrahydropyridine (VIII) by concentrated sulphuric acid, but other acidic reagents brought about a dimerization. The dimer contains nitrogen, is not acidic, and does not decolorize bromine; its structure was not determined, but may correspond to that of the "diacetonitrile" obtained from acetonitrile (4). Since no evidence of dimerization has been previously encountered in the reactions of delta ketonic nitriles, in this instance it may be related to the lack of a substituent group on the beta carbon atom. But γ-benzoylbutyronitrile, $C_6H_5COCH_2CH_2CH_2CN$, does not dimerize. This would tend to exclude such an explanation. However, this nitrile has a long aliphatic side chain, which allows of other possibilities and may be involved in the process.

The addition products (IX), (X), from cyanoacetamide and malonitrile were not studied in detail. The action of nitromethane was novel and unexpected—a "tetramolecular product" (XI) resulted. Evidently the intermediate addition products are still active enough to act again as addends. The tertiary nitro compound is relatively inactive and uninteresting. It is insoluble in alkalies as would be expected.


(IX)


(X)


(XI)

Experimental

A. The Addition Products

a. Methyl 1,5-dibenzoyl-3-cyanopentane-3-carboxylate (I). To a solution of 17 gm. of β-chloropropiophenone, 13 gm. of potassium acetate, and 80 cc. of hot methyl alcohol, from which the salt had been filtered, was added 5 gm. of methyl cyanoacetate, and, after making it permanently alkaline to moist litmus by use of a strong sodium methylate solution, the mixture was refluxed for half an hour. The addition product that separated after acidifying with acetic acid was filtered, washed with water, alcohol, and ether in order The yield was 70% if freshly prepared chloroketone had been used. The product is sparingly soluble in most of the usual organic solvents, but separates from acetic acid in fine needles, m.p. 144° C.* Calcd. for $C_{22}H_{21}O_4N$: N, 3.8%; mol. wt., 363. Found: N, 3.7, 3.7%; mol. wt., 387.

The amount of ester was varied until it became the solvent for the reactants, without affecting the result. The disemicarbazone, obtained in the usual manner, separated as a fine white powder, m.p. 220° C. with decomposition, insoluble in the common solvents; it was purified by simply washing with alcohol. Calcd. for $C_{24}H_{27}O_4N_7$: N, 20.5%. Found: N, 20.0%.

b. 1,5-Dibenzoyl-3,3-dicyanopentane (IX). By essentially the same procedure, malonitrile was added to vinyl phenyl ketone. On addition of the sodium methylate, a gelatinous precipitate was formed. After half an hour,

* *Melting points are corrected.*

water was added and the precipitate filtered. It formed glistening pointed rods from acetic acid, m.p. 215° C.; it is insoluble in the common solvents. Calcd. for $C_{21}H_{18}O_2N_2$: N, 8.1%; mol. wt., 330. Found: N, 8.0%; mol. wt., 318.

c. Ethyl 4-benzoylpyrazoline-5-carboxylate. This is not a trimolecular product but was prepared in connection with the problem. To a solution of vinyl phenyl ketone obtained from 5 gm. of β-chloropropiophenone, 4 gm. of potassium acetate, and 30 cc. of methyl alcohol, was added 5 gm. of diazoacetic ester, the mixture made faintly alkaline to litmus, and refluxed for four hours. When poured into water it formed a gum, from which 0.25 gm. of pyrazoline was isolated by treatment with methyl alcohol. It formed rosettes of fine rods, m.p. 140° C. Calcd. for $C_{13}H_{14}O_3N_2$: C, 63.5; H, 5.7%. Found: C, 63.1; H, 6.0%. This was the only procedure that gave anything definite; the use of a copper catalyst made no difference. The pyrazoline decomposed at 160° C. with much charring, and gave indeterminate products.

d. 1,5-Dibenzoyl-3-cyano-3-carbamylpentane (X).* From a similar solution of vinyl phenyl ketone and 2.2 gm. of cyanoacetamide, made alkaline with sodium methylate and refluxed for an hour, the addition product was isolated on acidification. It was recrystallized from acetone, and formed fine prisms, m.p. 200–201° C. Calcd. for $C_{21}H_{20}O_3N_2$: N, 8.1%. Found: N, 8.0%. Neither changing the time of heating nor using an excess of cyanoacetamide made any difference in the result.

Attempts to add benzyl or *p*-nitrobenzyl cyanides, and methyl phenylacetate to vinyl phenyl ketone were unsuccessful.

e. Nitrotri-(β-benzoylethyl) methane (XI). To a solution of vinyl phenyl ketone as in (*c*) was added 2 cc. of nitromethane, the mixture made alkaline and refluxed for two hours. On cooling, 3 gm. of the addition product separated. It was crystallized from hot methyl alcohol, from which it separated in long narrow plates, m.p. 132° C. It is insoluble in sodium hydroxide solution and the Skelly-solves†, moderately in alcohol and ether, and dissolves easily in other organic solvents. Calcd. for $C_{28}H_{27}O_5N$: N, 3.1%; mol. wt., 457. Found: N, 3.0%; mol. wt., 460.

B. Action of Hydrogen Bromide on (I)

a. Methyl 1,5-dibenzoyl-3-carbamylpentane-3-carboxylate (IV). A suspension of 10 gm. of the nitrile (I) in 50 cc. of acetic acid was saturated with hydrogen bromide without cooling; the solid all dissolved. After 16 hr. a nearly quantitative yield of the amide had separated; it was filtered, and recrystallized from a chloroform-methyl alcohol mixture. It formed white needles that tended to felt together, m.p. 224° C. The amide is sparingly soluble in acetic acid, practically insoluble in ether, the Skelly-solves, benzene, and carbon tetrachloride, but dissolves easily in chloroform. Calcd. for $C_{22}H_{23}O_5N$: C, 69.3; H, 6.0; N, 3.6%. Found: C, 69.6; H, 6.3; N, 3.6%.

* *The experimental work in this section was performed by Dr. F. B. Wells.*

† *The term "Skelly-solves" applies to various fractions of mixed hydrocarbons, marketed by the Skelly Oil Company, and replaces the indefinite terms "petroleum ether" and "ligroin", each of which needs to be accompanied by a boiling point range. Skelly-solve B, the one most commonly used in the laboratory, has a boiling point range of 60–68° C.*

Dehydration. A suspension of 1 gm. each of the amide and phosphoru pentoxide in 20 cc. of dry xylene was refluxed for half an hour, and the solu tion decanted from the gum. On cooling, needles of the nitrile separated m.p. 142° C., mixed melting point, 143° C.

Esterification. Attempts to esterify the amide were unsuccessful; either no reaction took place, or else intractable oils resulted. In one instance, a solid containing nitrogen, insoluble in alkali and not decolorizing bromine was obtained; it had an indefinite melting point, 144–148° C., after repeated recrystallizations from two solvents. It was soluble in the usual solvents except the ethers. Found: C, 73.0, 73.0; H, 5.0, 5.7; N, 4.1%.

1,5-Dibenzoyl-3-carbamylpentane-3-carboxylic acid. A mixture of 5 gm. of the ester amide, 2 gm. of potassium hydroxide, and 200 cc. of water was boiled for 15 min. and acidified with hydrochloric acid. The precipitate was filtered, and recrystallized from hot methyl alcohol; it formed needles, m.p. 280° C.; yield, 80%. On titration, the alkali consumed corresponded to a molecular weight of 363; calcd. for $C_{21}H_{21}O_5N$: 367.

b. The "Imide Bromide" (V). A suspension of 5 gm. of the addition product (I) in 25 cc. of chloroform was saturated with hydrogen bromide; after five hours the solid that had separated was filtered. It could also be isolated from the acetic acid solutions during the preparation of the amide. Although it does not evolve hydrogen bromide spontaneously, the latter was easily removed by solution in alcohol. It is insoluble in chloroform, acetic acid, ether, and the Skelly-solves, but dissolves in alcohol; the solution then deposits the above amide. The granular solid has a melting point of 165° C. with decomposition. Since it could not be purified, it was analyzed for bromine by shaking with an excess of standard alkali, followed by titration. Calcd. for $C_{22}H_{22}O_4NBr$: Br, 18.0%. Found: Br, 16.5%.

c. Methyl 2-bromo-3-(β-benzoylethyl)-6-phenyldihydropyridine-3-carboxylate (VI). The amide was filtered from an acetic acid solution prepared as in *(a)* after four hours, and the filtrate poured into water. The precipitated gum soon hardened, was removed, and crystallized from chloroform and methyl alcohol (small amounts from the alcohol alone). It formed diamond-shaped plates, m.p. 144° C. The bromopyridine is insoluble in ether, benzene, and carbon tetrachloride, but it dissolved readily in hot methyl alcohol, acetic acid, chloroform, and acetone. Calcd. for $C_{22}H_{20}O_3NBr$: C, 62.4; H, 4.6; Br, 18.8%; mol. wt., 426. Found: C, 62.0, 62.3; H, 4.4, 5.0; Br, 18.5, 19.0%; mol. wt., 430.

It decolorizes a bromine solution instantly. On titration 96% of the theoretical amount of bromine was taken up.

When the bromopyridine was dissolved in acetic acid and the solution was saturated with hydrogen bromide, the above amide deposited during the course of 18 hr. The same procedure gave some of the imide bromide, if chloroform was used as the solvent.

C. The Secondary Nitrile (III)

a. 1,5-Dibenzoyl-3-cyanopentane-3-carboxylic acid. The nitrile was easily hydrolyzed to this acid by shaking an ethereal solution of the nitrile with moist sodium methylate, but was more conveniently obtained by boiling for 15 min. a suspension of 6 gm. with 200 cc. of water containing 2 gm. of potassium hydroxide. After filtering if necessary, addition of a large excess of hydrochloric acid precipitated a gum, which became crystalline after several hours. The yield was 74%. It formed fine needles, m.p. 161° C., when recrystallized from dilute methyl alcohol. The molecular weight, found by titration, was 347 (calcd., 349).

b. Decarboxylation. The crude acid as prepared above was heated at 200° C. until gas evolution had practically ceased; 30 cc. of methyl alcohol was added to dissolve the gummy residue, from which 1.8 gm. (45% yield) of a white solid was obtained. It is important to heat for as short a time as possible. 1,5-Dibenzoyl-3-cyanopentane (III) is moderately soluble in hot methyl alcohol, from which it separates in rosettes of small rods, m.p. 100° C.; it is soluble in chloroform and acetic acid, and recrystallizes unchanged from acetyl chloride. Calcd. for $C_{20}H_{19}O_2N$: N, 4.6%. Found: N, 4.4%. In the Grignard machine it consumed three moles of reagent and evolved one mole of gas.

Monosemicarbazone. When treated in the usual manner, this diketone gave a monosemicarbazone, insoluble in most solvents; m.p., 202° C. with decomposition. Calcd. for $C_{21}H_{22}O_2N_4$: N, 15.4%. Found: N, 15.6%.

c. Action of acids. When treated with hydrogen bromide in acetic acid as above, this nitrile dimerized; the product was insoluble in alkali, and did not absorb bromine. It separated from chloroform-methyl alcohol mixtures in prisms, m.p. 265° C. Found: N, 4.6%; mol. wt., 550. The same substance was formed when the nitrile was heated with concentrated hydrochloric acid in a sealed tube, or when warming on the water bath with 50% sulphuric acid.

Concentrated sulphuric acid isomerized the nitrile to the tetrahydropyridine (VIII). A suspension of 2 gm. of the nitrile and 75 cc. of sulphuric acid was stirred for an hour—the solid slowly dissolved and the solution became a dark red, changing to green. The solution was then poured upon cracked ice and the precipitate extracted with chloroform, the extract treated in the usual manner and the residual solid crystallized from chloroform. The yield was 70%; m.p. 141° C. It formed microscopic plates insoluble in the ethers, but readily soluble in hot carbon tetrachloride or benzene, and also in chloroform, acetone, acetic acid, and ethyl acetate. Calcd. for $C_{20}H_{19}O_2N$: C, 78.7; H, 6.2%. Found: C, 78.8; H, 6.4%.

2-Keto-3-(β-benzoylethyl)-6-phenyltetrahydropyridine decolorized bromine instantly—the solution soon evolved hydrogen bromide and left a gum. Titration showed that one molecule was taken up. It was converted to a red pseudo solid by nitrous acid.

A trace of substance was isolated once only by the previous procedure (concentrated sulphuric acid). It did not decolorize bromine and was insoluble in chloroform. It crystallized from methyl alcohol in plates, m.p. 137° C. Calcd. for $C_{20}H_{21}O_3N_2$: C, 74.2; H, 6.5%. Found: C, 74.1; H, 6.2%.

C. Action of bromine. 2-Bromo-3-(β-bromo-β-benzoylethyl)-6-phenylpyridine (VII). The nitrile (III) (2 gm.) in 10 cc. of acetic acid reacted rapidly with 21 gm. of a 10% acetic acid solution of bromine. After 10 min. the mixture was poured into water containing a little sodium bisulphite, and the organic product separated, dissolved in chloroform, and worked up in the usual manner. On the addition of an equal volume of methyl alcohol and allowing the solution to stand overnight, the bromopyridine separated in large transparent six-sided plates, m.p. 151° C. A second crop was obtained by reworking the residues, the combined yield being nearly the theoretical. It is insoluble in the Skelly-solves, sparingly soluble in methyl alcohol, but readily dissolves in the other common solvents. Calcd. for $C_{20}H_{15}ONBr_2$: C, 53.9; H, 3.5; Br, 35.4%. Found: C, 53.8; H, 3.8; Br, 35.3%.

The bromine was completely removed by methyl alcoholic potash; potassium or silver acetates removed only one bromine atom (found by titration); in both cases the organic product was an intractable gum.

The bromopyridine was readily oxidized by permanganate or chromic acid, but the only recognizable product was benzoic acid. When the oxidation was interrupted or less reagent used, unchanged bromopyridine was found.

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FURTHER INVESTIGATIONS ON CATALYSIS BY LIQUID METALS[1]

By E. W. R. Steacie[2] and E. M. Elkin[3]

Abstract

A more detailed examination has been made of the kinetics of the methanol decomposition on solid and liquid zinc. The order of the reaction lies between zero and one-half on both solid and liquid. The heat of activation is approximately 30,000 calories per gram molecule. The reaction is mainly dehydrogenation.

Unsuccessful attempts to investigate certain other reactions on liquid metals are described.

Introduction

In a previous paper (5) the decomposition of methanol on solid and liquid zinc was described. The absence of a discontinuity in the rate of reaction at the melting point of zinc pointed to there being no marked differences in the mechanism of catalysis by solids and liquids. This cast some doubt on those hypotheses which ascribe catalytic activity to some one limited part of the surface of a solid.

Under the circumstances it is obviously desirable to make a more comprehensive investigation of catalysis by liquid metals. The present paper deals with a more detailed investigation of the decomposition of methanol on zinc, together with a number of attempts to investigate other reactions and other catalysts in a similar way.

Experimental Procedure

The method used was the same as that previously described. A sample of the metal catalyst was introduced into a Pyrex reaction vessel by distillation, or in some other way. The reaction vessel was connected to a capillary manometer, pumps, gauges, etc. Some of the reactant was let into the evacuated reaction bulb, and the rate of the reaction was inferred from the rate of change of pressure while the system was maintained at constant volume.

Experimental Results

I. The Decomposition of Methanol on Zinc

The Course of the Reaction

The methanol decomposition may proceed either by dehydrogenation or by dehydration (4), *i.e.*,

$$CH_3OH = H_2 + HCHO = 2\,H_2 + CO, \text{ or } 2\,CH_3OH = H_2O + CH_3OCH_3.$$

The first reaction would lead to a 200% pressure increase, while the second is not accompanied by a change in pressure.

[1] *Manuscript received April 19, 1934.*
Contribution from the Physical Chemistry Laboratory, McGill University, Montreal, Canada.
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Some pressure increases at completion observed at various temperatures and pressures were 142, 147, 147, 159, 128%. The reaction, therefore, proceeds mainly by dehydrogenation. The writers have used T_{25}, the time for the pressure to increase 25%, as a measure of the rate of reaction.

TABLE I

GASEOUS PRODUCTS OF THE METHANOL DECOMPOSITION

Temp., °C.	375	400	420	455
Absorbed by KOH (CO_2, HCHO, CH_3OH)	5.4	0.3	0.0	11.5
CO	28.7	32.9	32.4	30.8
CH_4	4.8	5.3	4.0	2.6
H_2	61.0	61.5	63.6	55.1
H_2/CO	2.13	1.87	1.96	1.79

Some typical analyses of the gaseous products of the reaction are given in Table I. It will be seen that these confirm the dehydrogenation mechanism.

It is noteworthy that the products of the decomposition on solid zinc are virtually the same as those from the reaction on liquid zinc.

The products are similar to those usually obtained from the pyrolysis of methyl alcohol. Thus a typical analysis (6) of the products from the decomposition in a Pyrex bulb at 570° C. is: CO, 34.6; CH_4, 2.9; H_2, 61.5%; H_2/CO, 1.78.

The Kinetics of the Reaction

It is rather difficult to make accurate inferences regarding the kinetics of the reaction, since the results are by no means highly reproducible. The main cause of this lack of reproducibility is the variation of the surface area of the catalyst, due to evaporation of zinc to the walls of the bulb. After a series of runs it is often found that there is an appreciable deposit on the walls of either small crystals or droplets of zinc, depending on the temperature of the system.

This evaporation to the walls is erratic, and depends on (*a*) the presence of temperature gradients along the furnace during heating or cooling, and (*b*) on the vacuum treatment, presence of traces of air, etc. It often happens that on a given catalyst, maintained at constant temperature, some 8 or 10 runs will show excellent agreement. There will then be a sudden change in the activity of the catalyst, and the next 10 runs will agree with one another but not with the first set. However, it is usually possible to obtain definite indications of the effect of various factors on the kinetics of the process from groups of consecutive runs.

Series I

To determine the effect of alcohol concentration on the rate, a series of runs were made at 374° C. with varying pressures of alcohol. The catalyst consisted of a zinc button having an area of about 3.0 cm^2. It was prepared by distilling zinc shot which had been melted in an atmosphere of hydrogen. It was then left in contact with hydrogen for 12 hr. at 500° C.

TABLE II

SERIES I

Run no.	Initial press., cm.	T_{20}, min.	Run no.	Initial press., cm.	T_{20}, min.	Run no.	Initial press., cm.	T_{20}, min.
1	4.70	15.0	6	2.80	6.1	11	1.40	4.6
2	10.20	28.0	7	6.45	10.5	12	7.60	12.2
3	10.50	25.0	8	8.35	15.3	13	8.30	9.1
4	10.65	23.8	9	10.45	15.0	14	7.80	10.0
5	4.35	14.0	10	10.60	14.1			

NOTE:—*Temp., 374.5° C. Surface area = 3 cm².*

The results are given in Table II, and are plotted in Fig. 1.

It will be seen from Fig. 1 that the results fall into two groups, *viz.*, Runs 1–5, and 6–14. The variation of rate with pressure is approximately the same in each group, but a sharp change in the activity (surface) of the catalyst has occurred between Runs 5 and 6.

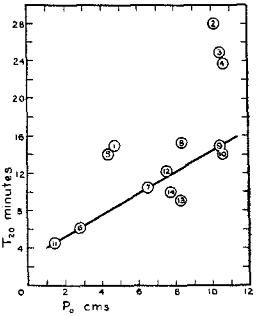

FIG. 1. *Series I*

Series II

The catalyst used was a museum sample of "Spectroscopically Pure Zinc" donated by the Research Division of the New Jersey Zinc Co. It had been used previously for a few runs with methyl alcohol, and with ethylene and hydrogen. It was placed in the reaction vessel without any purification. The surface area was about 5 cm². This series of runs were made at a temperature of 374.5° C. The results are plotted in Fig. 2.

Series IV

Catalyst III was used at 436° C. for about eight runs, and was then cooled to 374.5° C. for Series IV. The results are given in Fig. 3.

From a comparison of Series I, II, and IV, it is apparent that the effect of pressure on the rate of reaction is approximately the same in all cases. The results are not sufficiently reproducible to enable an exact calculation of the order of the reaction, but it evidently lies between 0 and 0.5

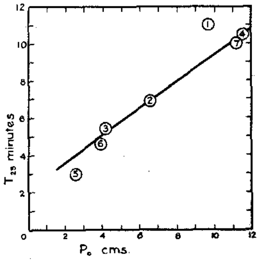

FIG. 2. *Series II*

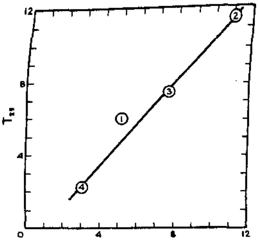

FIG. 3. *Series IV*

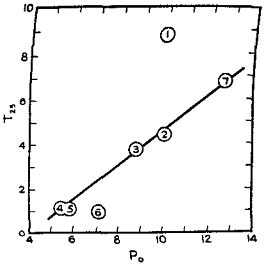

FIG. 4. *Series III*

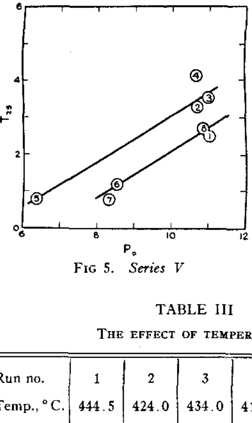

FIG 5. *Series V*

Series III

The catalyst was prepared by distillation. It was removed, cleaned by scraping, sealed into a new reaction bulb, and used without reduction. This series of runs were made at 436.1° C. (above the melting point of zinc). The data are plotted in Fig. 4.

Series V

After Series III the catalyst was used at 374.5° C. (below the melting point) for Series IV, and then heated to 436.1° C. again and used for Series V. The results are given in Fig. 5.

Taking Series III and V together, it is evident that the order of the reaction at 436.1° C. is about the same as that at 374.5° C., although in the one case we are dealing with solid, and in the other case with liquid zinc.

TABLE III

THE EFFECT OF TEMPERATURE

Run no.	1	2	3	4	5	6
Temp., ° C.	444.5	424.0	434.0	412.0	404.0	376.0
T_{25}, min.	4.13	7.58	6.08	12.12	16.25	23.33

NOTE:—*Initial pressure, 10.25 cm.*

Series VI

The catalyst was a fresh sample of "Spectroscopically Pure Zinc". A series of runs were made at different temperatures. The results are given in Table III.

If ln T_{25} is plotted against the reciprocal of the absolute temperature, a good straight line is obtained, from the slope of which the heat of activation is found to be 30,900 calories per gram molecule. If we treat the data given

in the previous paper in the same way, we obtain 29,100 and 29,400 calories per gram molecule respectively from Figs. 3 and 4. Hence the mean value is 29,800 calories.

One of the best investigations of the temperature coefficient of the alcohol decomposition is that of Dohse (1) who worked with unimolecular layers on zinc oxide catalysts. He obtained 22,500 calories for the heat of activation of the first step in the dehydrogenation, and 35,000 calories for the subsequent decomposition of formaldehyde. The writers' result for the over-all process is therefore in general agreement with his. There is, of course, no reason why the heats of activation should be identical on different catalysts, but the general agreement indicates that the process is proceeding in a normal way on the writers' catalysts.

Discussion

In the previous paper it was shown that there was no abrupt change in the rate of decomposition of methyl alcohol on zinc at the melting point of the metal. Since all crystal imperfections, grain boundaries, etc., disappear on melting, this observation throws considerable doubt on those theories of catalytic action which ascribe the activity of the catalyst to some one limited part of the surface.

The present investigation strengthens the conclusions previously drawn, since it shows that not only is there no discontinuity in rate at the melting point of the catalyst, but in addition the kinetics of the process and the products formed are fundamentally similar above and below the melting point. It is therefore very improbable that there is any surface structure peculiar to crystalline solids which is responsible for catalytic activity in general. It is, of course, possible that in certain special cases the entire surface may not be uniformly active. These conclusions are supported by the recent work of Maxted and his collaborators (2, 3). By progressively poisoning platinum catalysts and determining the activation energies of certain reactions on the poisoned catalysts, they have demonstrated the "energetic homogeneity" of the surface.

II. Other Reactions on Liquid Metals

(a) The Hydrogen-Ethylene Reaction on Zinc

An attempt was made to investigate the hydrogen-ethylene reaction on a liquid zinc surface. The zinc was prepared as in the previous experiments with methyl alcohol. The surface area was about 5 cm^2. No reaction was obtained up to 462° C. with a 2 to 1 hydrogen-ethylene mixture.

The main difficulty in this work is the necessity of using very small surfaces. On this account it is impossible to investigate a reaction unless the catalyst is extremely effective. As a result very few reactions can be successfully investigated. This is not due to any lack of efficiency characteristic of the liquid state. Thus it was found that a sample of smooth copper foil having

the same area as the zinc sample used above was also incapable of effecting any measurable hydrogenation of ethylene.

(b) Reactions on Solid and Liquid Tin

Attempts were made to investigate the hydrogenation of ethylene and the decomposition of methanol on tin. In each case the catalytic reaction was negligible up to temperatures at which either a heterogeneous reaction on the Pyrex walls, or a homogeneous reaction, set in. In both cases a series of copper-tin alloys containing up to 5% of copper were also ineffective.

(c) Reactions on Solid and Liquid Cadmium

Attempts were made to investigate the decomposition of methanol and of methyl formate on cadmium. This proved to be impossible on account of the high vapor pressure of cadmium which caused a large amount of distillation to the walls, and invariably plugged the capillary tube leading to the manometer.

Conclusions

It is apparent that the main difficulty in this work is the very small surface area of the catalyst compared with the large surface of the walls of the reaction vessel. As a result it is only possible to investigate a few reactions in which the catalyst is exceptionally effective and the walls are exceptionally ineffective. In order to carry out successfully an extensive investigation of catalysis by liquid metals, it is essential that this difficulty be overcome. Investigations are therefore being carried on which involve entirely new experimental methods.

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THE EFFECT OF ATMOSPHERIC SULPHUR DIOXIDE ON COTTON TEXTILES[1]

By O. M. Morgan[2] and B. J. Kenalty[3]

Abstract

The effect of drying, in air containing one and two parts of sulphur dioxide per million, cotton fabric wetted with solutions such as those which it would encounter in power laundry rinse waters has been investigated. Factors such as sulphur dioxide concentration, humidity, temperature and light have been examined and kept under all possible control. The sulphur dioxide has been found to have little or no effect in producing deterioration of the fabric.

Introduction

The term "winter damage" has been used to designate a somewhat obscure kind of deterioration occurring in laundered fabrics. It first attracted attention in New England and appears to occur in laundered fabrics which have been repeatedly dried, particularly during the winter, in the atmosphere of industrial cities, especially those located on or near the coast.

Winter damage is found only in wet-wash fabrics which are returned from the laundry to the customer in a damp condition and which are hung outside to dry. It is at this time that sulphur dioxide may be absorbed by the fabric. Winter damage is very seldom found to occur in fabrics that are completely finished and returned dry to the customer.

It has been supposed that the agent responsible for winter damage is atmospheric sulphur dioxide which is absorbed by the fabric, especially in the winter, when temperatures are lower and the concentration of atmospheric sulphur dioxide in general is higher than at other seasons. It is supposed that the sulphurous acid formed in the fabric is, if suitable agents are present, oxidized to sulphuric acid, and that the latter, although present in only small amount, becomes highly concentrated at the time of ironing and causes the formation of degenerated cellulose and consequent weakening of the fabric.

Previous Work

If the above theory as to the cause of winter damage is the correct one it should not be difficult to produce this damage artificially in the laboratory. One attempt on record is that by Wilkie (5) in which cotton towels were doped with various solutions and allowed to dry in a cabinet containing sulphur dioxide, the initial concentration of which was 1 part per million (p.p.m.). No control of this concentration was maintained, hence the effective concentration would be much lower than 1 p.p.m. owing to absorption of the gas by the fabric and by condensed moisture on the walls of the cabinet. The towels were removed once daily, ironed at 438° F., moistened with

[1] *Manuscript received May 25, 1934.*
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distilled water, and returned to the cabinet. No intermediate laundering was performed and the free acid and sulphates were allowed to accumulate.

The tensile strength losses were extremely high, in some cases ranging from 90 to 100% with as few as 17 treatments. This, to the present writers, hardly seemed possible when the concentrations of sulphur dioxide are considered.

It should be pointed out that in Wilkie's experiments the samples after immersion in the solutions were not, as is the practice in power laundry work, centrifuged in order to remove excess liquid, and it is not clear whether in all cases they were even wrung out by hand; further, the period of fumigation after each treatment was 24 hr. In the present work all excess liquid was extracted from the samples by centrifuging and the samples were fumigated only until dry (3 to 4 hr.). This procedure approaches more closely than Wilkie's to conditions in actual practice.

Certain further features of Wilkie's experiments should be noted. In the first place, it should be remarked that there were no control samples treated in all respects similarly to the fumigated samples, except for exposure to sulphur dioxide. In the second place the samples were not subjected to actual laundering and hence the results give no direct measure of the effect of drying in air containing sulphur dioxide after laundering. In the third place, the concentrations of the doping solutions were greater than those of the corresponding solutions used in laundry practice, as Table I shows.

TABLE I

STRENGTH OF SOLUTIONS APPLIED TO FABRICS

Supply	Concentration used by Wilkie (5), % Cl	Concentration found in practice, % Cl
Sodium hypochlorite	0.045	0.01
Sodium hypochlorite	0.025	0.01
Soap	0.25	0.10
Acetic acid	2.0	0.05

Hence if any of the materials listed in Table I did accelerate the oxidation of sulphurous to sulphuric acid their effect might be greatly enhanced by the overdosage in the doping of the test fabrics. Iron, sodium chlorate and calcium bicarbonate were also used in solution as doping agents.

Experimental Work

Apparatus

In the present attempt to produce winter damage artificially, the doped fabrics were hung on shellacked metal rods in an insulated fumigation cabinet measuring 6 ft × 5 ft. × 3 ft. 2 in. internally. Air was forced through the cabinet by means of a blower. Sulphur dioxide was injected into the air stream from a cylinder of the liquid gas through a modified pressure-differential flowmeter capable of measuring an injection difference of two bubbles per minute. The air and sulphur dioxide passed through a long rectangular mixing tank fitted with baffle plates and thence to the top of the fumigation

cabinet where they were further mixed in the distribution chamber. This chamber was so arranged that the gas was introduced into the cabinet through nine symmetrically placed openings, each 3 in. in diameter, assuring an even draught through the cabinet. Nine similar openings were placed in the bottom of the cabinet leading into a collection chamber which had a lead off to the hood vent.

The content of sulphur dioxide in the incoming and the outgoing air was measured by means of an apparatus essentially similar to the automatic recorder described by Thomas (2, 3, 4). By means of a two-cell automatic aspirator, gas samples were taken alternately from the inlet gas in the distribution chamber and from the off-gas in the collection chamber, the period of aspiration in each case being 20 min. The volume of the gas sample was measured by means of a Sargent wet test meter. The gas was aspirated through a dilute solution of hydrogen peroxide which converted it to sulphuric acid. The electrolytic conductivity of the sulphuric acid solution was recorded

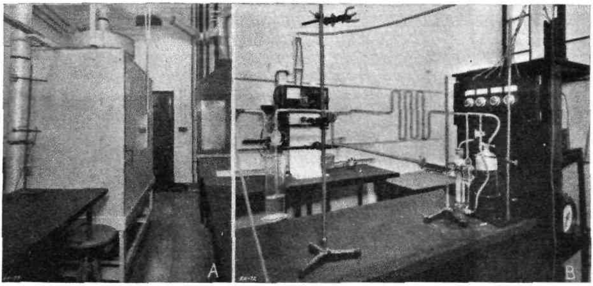

FIG. 1. *Views of fumigation apparatus.* A *shows the cabinet with header on top and exit pipe on left. The incoming and the outgoing air are sampled through the glass tubes shown. A mixing chamber on the left of the cabinet is not visible in the photograph.* B *shows the flowmeter device by which bubbles of sulphur dioxide are introduced into the ingoing air from a cylinder of the gas. On the right, only partly visible, is the recorder which, in conjunction with the automatic recording Wheatstone bridge (on wall), measures the sulphur dioxide content of the ingoing and outgoing air of the cabinet.*

by means of a Leeds and Northrup conductivity recorder. The conductivity of the solutions was calibrated in terms of the concentration of the sulphur dioxide passing through the fumigation cabinet. Hence a running check was maintained on the sulphur dioxide concentration in the gas stream. By means of this recording equipment, concentrations of sulphur dioxide of 0.05 p.p.m. could be recorded. All runs were made at either 1 or 2 p.p.m. of sulphur dioxide by volume.

Arrangements were made for placing a humidifier at various positions in the air stream so that relative humidities up to 95% could be obtained and maintained within 5% of any value required. Air speeds through the system

were determined by means of a calibrated anemometer. The temperature in the fumigation cabinet was dependent upon outside weather conditions. All heat was shut off from the room in which these experiments were conducted in order to reduce the temperature and thus induce as much sulphur dioxide absorption as possible. A recording hygrothermograph was used to record temperature and humidity data. This instrument was calibrated every seventh day.

The various doping agents applied to the test pieces of fabric were used in the concentrations shown in Table II. Where several agents were used in combination they were each present in the concentration noted in the table. The fabrics were soaked in these solutions for 10 min., centrifuged in a porous basket for 10 sec. at a constant rate to remove excess moisture and prevent dripping, and then placed in the fumigation cabinet until thoroughly dry.

TABLE II

Agents applied to the test pieces

Agent	Concentration*
Iron	0.025% ferric chloride followed by 0.1 sodium carbonate according to Wilkie's method
Soap	0.005%
Bleach	0.005%
Oxalic acid, Acetic acid	Sufficient to reduce the pH to 5.5
Ammonium carbonate	0.1%
Sucrose	0.1%

* *The concentrations of soap, bleach and acids correspond to rinsing concentrations in a power laundry.*

Experimental Results

The fabric used in these experiments was a good grade of cotton sheeting with a thread count of 82 × 68. The original tensile strength of the warp threads was 53.1 lb. per in. of width. Tensile strength measurements were made with an Alfred Suter tensile strength machine having 1-in. jaws, 3 in. between the jaws, and a lower jaw travel of 12 in. per min. The "strip" method for tensile strengths was used.

Laundering was performed in a local laundry which adheres rigidly in washroom practice to the recommendations of the Laundryowners National Association. Soap and soda ash were the washing agents used and the loads of clothes were acidified to a pH of 5.5 with a mixture of sodium acid fluoride and sodium silico fluoride in the last rinse. The washing formula consisted of four suds baths and six rinse baths (1).

Experiments Nos. 1 and 2

Three sets of fabrics were used in each of these experiments to determine the effects of various agencies. Their treatments may be summarized as follows:

Set No. 1. These fabrics were treated with their respective doping agents, dried in the cabinet in air containing sulphur dioxide, ironed at about 400° F., aged for three hours, laundered in a power laundry and the cycle repeated.

Set No. 2. The fabrics were treated with their respective doping agents, dried in the laboratory at room temperature, but given no treatment with sulphur dioxide, ironed at about 400° F., aged for three hours, laundered with Set No. 1, and the cycle repeated.

Set No. 3. The doping treatment was applied as usual, followed by fumigation and ironing at about 400° F. These fabrics were then allowed to hang in the laboratory until the next morning when the cycle was repeated. This set was not laundered.

Hence, Set No. 1 was used to determine the combined effects of sulphur dioxide treatment plus laundering; Set No. 2 was used to determine the effect of laundering alone; and Set No. 3 to examine the effect of sulphur dioxide alone, treatment with various agents being common to all sets. Tables III and IV show the conditions prevalent in Experiments Nos. 1 and 2 and the losses in tensile strength.

TABLE III

EXPERIMENT NO. 1. EFFECT OF 1 P.P.M. SULPHUR DIOXIDE

Doping agent	Losses in textile strength after 40 consecutive treatments, %		
	Set No. 1, laundered and fumigated	Set No. 2, laundered only	Set No. 3, fumigated only
Distilled water	20.4	15.6	6.8
Iron	21.6	21.2	8.7
Bleach	24.8	21.4	8.3
Soap and bleach	25.6	24.4	9.6
Soap and bleach and oxalic acid	28.6	27.2	12.4
Soap and bleach and acetic acid	28.8	30.9	7.9
Ammonium carbonate	23.0	20.0	1.0

NOTE: *Concentration of sulphur dioxide, 1 p.p.m.; temperature in fumigation cabinet, 6.5–14° C.; relative humidity in fumigation cabinet, 50–60%; air flow through fumigation cabinet, 220 cu. ft. per min.; total time of exposure, 130.1 hr.; dates of experiments, December 7, 1932, to February 9, 1933.*

It will be noted that the normal loss in tensile strength due to laundering is 15.6%. Where doping agents other than distilled water were used the losses were higher, particularly when acids or oxidizing agents were present. In Set No. 3 the losses are very much lower than those obtained by Wilkie (5). Further discussion will be found in a later section.

In all experiments tensile strength measurements were made after 10, 25, 40 and 50 treatments. Only the results obtained after the complete series of treatments in any one experiment are, however, given here, as it was felt that, owing to the irregularities inevitably encountered in the results of experiments of this kind, only clearly marked effects can be regarded as significant.

TABLE IV

EXPERIMENT No. 2. EFFECT OF 2 P.P.M. SULPHUR DIOXIDE

Doping agent	Losses in tensile strength after 50 consecutive treatments, %		
	Set No. 1, laundered and fumigated	Set No. 2, laundered only	Set No. 3, fumigated only
Distilled water	21.6	22.0	5.9
Iron	24.0	20.2	0.0
Bleach	32.4	29.9	3.8
Soap and bleach	34.2	27.6	13.4
Soap and bleach and oxalic acid	28.1	29.2	9.0
Soap and bleach and acetic acid	30.6	31.4	10.4
Ammonium carbonate	23.4	20.4	0.9

NOTE: *Concentration of sulphur dioxide, 2 p.p.m.; temperatures in fumigation cabinet—(a) February 15 to April 7, 1933, 12–14° C.; (b) April 17 to May 2, 1933, 18–23° C.; total time of exposure, 166.0 hr.; dates of experiments, February 15 to May 2, 1933; other conditions were the same as in Experiment No. 1.*

Experiment No. 3

In experiment No. 2 it was noted that the rate of loss of tensile strength decreased after the weather became warmer (April 7, 1933) and the temperature in the fumigation cabinet rose. To investigate the effect of temperature change, experiment No. 3 was conducted in summer weather. The concentration of sulphur dioxide was 1 p.p.m. The data obtained are shown in Table V (cf. results of experiment No. 1).

TABLE V

EXPERIMENT No. 3. THE EFFECT OF HIGHER TEMPERATURES IN THE FUMIGATION CABINET

Doping agent	Losses in tensile strength after 50 consecutive treatments, %		
	Set No. 1, laundered and fumigated	Set No. 2, laundered only	Set No. 3, fumigated only
Distilled water	15.4	14.5	0.0
Soap and bleach and acetic acid	19.0	20.8	3.6
Ammonium carbonate	19.0	12.2	1.9
Sucrose	17.8	16.2	5.6

NOTE: *Concentration of sulphur dioxide, 1 p.p.m.; temperature in fumigation cabinet, 24–26° C.; relative humidity in fumigation cabinet, 55–65%; total time of exposure, 197.1 hr.; dates of experiments, June 6 to August 18, 1933.*

On comparing experiments Nos. 1 and 3 it will immediately be seen that higher temperatures reduce the losses in tensile strength considerably. However, in none of the three experiments were the losses suffered of the order of those obtained by Wilkie (5).

TABLE VI

EXPERIMENT NO. 4. EFFECT OF SLOW DRYING IN THE PRESENCE OF HIGH HUMIDITIES

Doping agent	Sulphates, %*	Losses in tensile strength after 50 consecutive treatments, %	
		Blanks†	Treated samples
Distilled water	0.097	2.0	7.5
Iron	0.138	2.8	22.1
Soap and bleach and acetic acid	0.070	4.8	7.9
Ammonium carbonate	0.092	1.5	1.9
Sucrose	0.104	3.0	5.4

* *Percentage of sulphates is calculated on the basis of sulphuric acid.*

† *Blanks were damped and ironed along with the other samples, but were not admitted to the fumigation chamber.*

NOTE: *Concentration of sulphur dioxide, 2 p.p.m.; temperature in fumigation cabinet, 5–15° C.; relative humidity in fumigation cabinet, 80–90%; air flow through fumigation cabinet, 78 cu. ft. per min.; total time of exposure, 578.7 hr.; dates of experiments, December 4, 1933, to January 10, 1934.*

Experiments Nos. 4 and 5

Three factors remained to be investigated, namely, high humidities, slow rates of drying, and the effect of light. In experiment No. 4 a run was made using slow drying in the presence of high humidities; in experiment No. 5, slow drying, high humidities and ultra-violet light from a General Electric Type S1 sun lamp were employed. In the latter experiment the doped fabrics were hung on a circular support 28 in. in diameter, with the sun lamp in the centre of the circle. In neither of these experiments was laundering applied, and free acid and sulphates were allowed to build up in the fabrics according to the method used by Wilkie (5). The results of these experiments are shown in Tables VI and VII.

TABLE VII

EXPERIMENT NO. 5. EFFECT OF COMBINED SLOW DRYING, HIGH HUMIDITIES AND ULTRA-VIOLET IRRADIATION

Doping agent	Sulphates, %	Losses in tensile strength after 50 consecutive treatments, %
Distilled water	0.136	4.4
Iron	0.155	13.3
Soap and bleach and acetic acid	0.177	6.9
Ammonium carbonate	0.336	12.2
Sucrose	0.166	7.9

NOTE: *Concentration of sulphur dioxide, 2 p.p.m.; type of light used, G.E. Type S1 sun lamp; total time of exposure, 567.5 hr., including sun lamp exposure of 75.0 hr.; distance of lamp from samples, approximately 14 in.; dates of experiments, January 15 to February 20, 1934; other conditions were the same as in Experiment No. 4.*

Discussion of Results

No mention has been made thus far of the reasons for using ammonium carbonate and sucrose as doping agents. From a theoretical standpoint these should be effective inhibitors of sulphuric acid formation. The slight alkalinity of ammonium carbonate should be sufficient to neutralize any acid

that may be formed. Ammonium sulphate would be formed, but it would be harmless to the fabrics. It is also well known that a trace of sucrose in a water solution of sulphur dioxide will inhibit any oxidation to sulphur trioxide; one analytical procedure for the determination of sulphur dioxide makes use of this fact.

In Wilkie's experiments *practical* or *complete destruction* of the fabric was caused by drying in air containing 1 p.p.m. of sulphur dioxide, after immersion in a solution of soap, bleach and acetic acid (concentrations given in Table I) and repeating this 17 times. The same effect was produced by 30 similar treatments with iron, while profound deterioration was produced by 17 treatments with bleach and soap followed by fumigation; even fumigation following mere wetting with distilled water reduced the tensile strength by 40%, and brought the sulphate content of the fabric to 0.59% after 50 treatments. The limitations and deficiencies of these experiments have already been pointed out.

In the present experiments, even when the sulphur dioxide content of the air was 2 p.p.m., no similar deterioration has been observed. Indeed, after allowing for the experimental error inevitably attaching to experiments of this kind on textiles, it must be concluded that the experiments offer no clear evidence of *any* deteriorating action of atmospheric sulphur dioxide when damp cotton textiles are dried in it. Even when, as in the present experiments, residual bleach, soap and sour are deliberately applied to the fabric after laundering, drying in air containing sulphur dioxide produces only an insignificant amount of deterioration or no deterioration at all. Much less, then, is it likely that drying in such an atmosphere will produce deterioration when the fabric is merely laundered and rinsed.

In the present work, humidity, temperature, two concentrations of sulphur dioxide, the effect of light, and the effect of common laundering supplies have been studied in an effort to produce sulphur dioxide damage in cotton fabrics. Two parts per million of sulphur dioxide is much in excess of the concentration found in the atmosphere of most manufacturing cities. All possible variables in these experiments have been rigidly controlled. The results show that it has not been possible to produce serious damage with such concentrations. Even in experiments Nos. 4 and 5, in which air movement through the fumigation cabinet was very slow and temperatures were low and humidities were high, the fabric was not seriously damaged. These conditions are considered to be ideal for the development of winter damage.

It is not claimed that this is the last word in this connection nor has the cause of the damage, generally designated as winter damage, been established. It is desired, however, to put these results on record as an aid to future experimenters who may care to investigate this field. There is a possibility that polluted atmospheres in manufacturing cities may contain certain amounts of sulphur trioxide which would be directly damaging, even in much smaller quantities, than would sulphur dioxide. In many investigations of atmospheric pollution this point has not been considered.

Acknowledgments

The writers wish to express their appreciation to Vail's Laundry, Ottawa, for laundering the test fabrics; and also to Mr. W. C. Davis of these laboratories for the sulphate analyses.

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THE RELATIVE RIGIDITY OF WELDED AND RIVETED CONNECTIONS[1]

By C. R. Young[2] and K. B. Jackson[3]

Abstract

Little quantitative information being hitherto available concerning the degree of restraint developed at the ends of steel beams and girders with welded connections, and the extent of possibly related economics and improvements in frame stiffness being correspondingly uncertain, the experimental investigation of two distinct aspects of joint rigidity was undertaken. These were:

(a) The relative capacity under gravity load of beam-girder and beam-column connections for developing beam restraint, or continuity, designed with a view to reducing the required weight of the connected beam.

(b) The relative values of certain typical connections in resisting the deformation of a frame due to lateral loads, either in one direction or subject to reversal.

Two series of specimens were fabricated and tested in relation to plain rating beams. The first series, designed for the purpose of studying beam continuity, consisted of ten 9-in., 20.5-lb. Bethlehem beam double-cantilever specimens, employing five different types of welded connections. The second, designed primarily for the study of wind bracing rigidity, consisted of twelve 18-in., 47-lb. Carnegie beam double-cantilever specimens with three different types of connections. These types included the welded T, the welded gusset and, for comparison, the riveted T. For half the specimens of the second series the connections were to a central transverse plate simulating the web of a column, and for the other half to the flanges of a 12 by 12-in., 110-lb. H-column.

Coefficients of restraint, that is, the ratio of actual to 100% restraint, were ascertained by determining end slope angles with the aid of a special type of longitudinal extensometer attached to the beam flanges. Although, for convenience, the specimens were tested as double-cantilevers the results were readily transformed to be applicable to the partially restrained beams simulated by the specimens.

Story drift angles, that is, the angle through which the columns of a building subjected to horizontal force would tilt in one story of height due to the deformation of the connections, were ascertained by utilizing the end slope angles mentioned above and the observed deflections of the specimens. The results for the specimens were transformed by calculation to be applicable to a 20-ft. bay of an actual building.

Broadly stated, the results obtained in the study of the connections tested were:

(1) Welded connections as ordinarily designed for end restraint fall short of the ideal value of 0.75 for the coefficient of restraint by from 10 to 25%.

(2) Both welded and riveted connections designed primarily for capacity wind moment develop a coefficient of restraint in excess of the most economical value (0.75) required to resist gravity loads only.

(3) A beam welded to a column web may develop less story drift angle than would a plain beam integral with the column. T's riveted to a column flange may, for a 20-ft. bay, give rise to a lineal drift of as much as 0.36 in. per 12-ft. story under a capacity wind in one direction, while welded T's under the same conditions showed 0.09 in. and welded gussets only 0.03 in.

(4) In the connections designed for beam continuity only, the compression attachment was in all cases more yielding than the tension one. Transfer of axial compression by longitudinal welds to the edges of a thin flange introduces much yield due to horizontal shear in the flange.

(5) In wind connections either to a column web or to a column flange, the top and bottom attachments, under capacity reversing load, gave approximately equal total longitudinal deformations for each of the three types.

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[3] *Assistant Professor of Engineering Physics, University of Toronto.*

(6) From a third to a half of the flexural slope in a connection to an unstayed column flange may be charged to the deformation of the column flange.

(7) In wind connections to an unstayed column flange the welded connections gave regional flexural slopes from 17 to 63% of the corresponding ones developed in the riveted T-connections, depending on the region, or longitudinal zone. Consequently, the welded connections were definitely stiffer than the riveted ones in all regions.

(8) The aggregate longitudinal elastic deformation in tension for capacity reversing load was greater than the aggregate deformation in compression, the maximum excess being 70%. This was for the riveted T-connection to a column web.

(9) For the riveted T's a large part of the longitudinal deformation was non-elastic slip. It amounted to 31% for the connection to a column web and 46% for a connection to a column flange.

(10) Welding of stiffeners to the column flanges reduced the longitudinal deformation of the welded T and the welded gusset connections to 75 and 85% of their previous values, respectively.

(11) For the specimens designed for beam continuity only, the ratios of the test factor of safety to the design factor of safety, based on an estimated ultimate strength of 10,000 lb. per lineal inch of $\frac{3}{8}$-in. fillet weld with uniform distribution of stress, varied from 0.65 to 0.97.

(12) For the specimens designed primarily as wind connections, the ratio of test factor of safety to design factor of safety, based on assumed uniform stress distribution, varied from 0.88 to 0.98 for the riveted specimens, from 0.49 to 0.83 for the welded T-specimens and from 1.06 to 1.29 for the welded gusset plate specimens. It is thus evident that non-uniformity of stress, which obviously existed to a marked degree for the welded T-connections, may cause the capacity of certain types of connections to fall considerably below that estimated on the basis of uniform distribution.

I. Introduction

1. *Nature of the Problem*

In the problem undertaken two distinct aspects of joint rigidity in steel construction were investigated experimentally. These were:

(a) The relative capacity under gravity load of beam-girder and beam-column connections for developing beam restraint, or continuity, designed with a view to reducing the required weight of the connected beam.

(b) The relative values of certain typical connections in resisting the deformation of a frame due to lateral loads, either in one direction or subject to reversal.

(a) It is well known that the weight of steel beams may be substantially reduced if a restraint be introduced at the supports comparable to that secured as a matter of course in properly constructed reinforced concrete beams resting on several supports. Nevertheless, so little information has been available regarding the actual degree of restraint developed that specifications, with few exceptions, ignore it altogether. While this practice contributes in an indefinite manner to the factor of safety of the connected beam itself, it disregards what may be large moments in the connections, moments that exist whether advantage is taken of them or not in proportioning the beam. It is consequently desirable to ascertain the degree to which restraint is actually developed and to what extent its development is advantageous. With welded end-connections it has been possible to attain a high percentage of restraint, but it will be shown that, for gravity loads, the ideal restraint is considerably less than the maximum attainable.

(b) Growing out of the need for minimizing the deflection and vibration of tall steel buildings under wind force, much attention is now being directed to the rigidity of wind connections, apart from their strength or factor of safety under load. Although welded wind connections have, up to the present, not been extensively employed for heavy wind duty, it has appeared that their use might impart to a building a rigidity greater than that hitherto attained by the use of riveted connections. So far as the development of maximum rigidity against lateral forces is concerned, manifestly the ideal restraint in connections is the maximum attainable.

II. Experimental Work

2. *Classes of Specimens*

Two series of specimens were fabricated and tested. The first, or preliminary series, fabricated in 1929, consisted of light members and welded connections, and served to develop the method of procedure and to indicate the relative merits of the various types of connections used. This series was designed for the study of beam continuity only.

The second series, fabricated in 1930, consisted of relatively heavy members and included both riveted and welded connections. Though this series was primarily designed for the study of wind-connection rigidity it also served to extend the study of beam continuity.

3. *Specimens of the First Series*

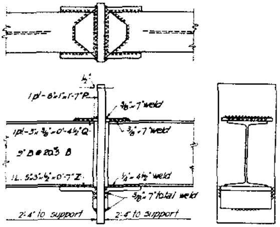
In view of the fact that typical steel girders in building floors will commonly range from 15 to 24 in. deep and be 18 to 22 ft. in span, with a total load of perhaps 60,000 to 80,000 lb., it would manifestly be impracticable to test many such full-size members under conditions duplicating those found in the floor. It was therefore decided to design double-cantilever specimens which represented the condition in a real floor as accurately as possible, but, in order to keep the size of the specimens within reasonable bounds, to base them on a floor panel with dimensions and loading per unit of floor area about 70% of those obtaining in a typical floor. The specimens were consequently so devised that under working stresses in the connections, the maximum moment would be the same as at the supports of a perfectly restrained, uniformly loaded, beam of 14 ft. span carrying a total load of 24,000 lb. This reduced the size of the required test beams to 9-in., 20.5-lb. Bethlehem I's.

Five types of welded connections, involving variations in the tension connection only, *A*, *B*, *C*, *D* and *E* were designed, as indicated in Fig. 1.

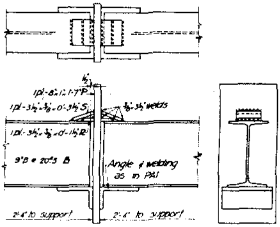
Two "beam" specimens, *BA*1 and *BA*2, for purposes of comparison, and 10 "plate" specimens, *PA*1, *PA*2, *PB*1, *PB*2, *PC*1, *PC*2, *PD*1, *PD*2, *PE*1 and *PE*2, representing the connection of beams to the web of a girder or column and incorporating duplicate pairs of the various connections, were fabricated as indicated in Fig. 3 and tested as described below.

In the design of these specimens a working stress of 18,000 lb. per sq. in. was employed for the beams and the connection material apart from the

welds. For the latter a design stress of 5,000 lb. per lineal in. of $\frac{3}{8} \times \frac{3}{8}$ in. fillet weld was employed giving a nominal factor of safety of about 2.0 under a specimen load of 24,000 lb. The weld stress was fixed rather high in order to ensure failure in the welds rather than in the material. Actually, horizontal shear stresses in the compression flanges of the beam adjacent to the shelf angle welds proved excessive, producing unexpectedly large deformation in the compression connection.



SPECIMENS PA1 & PA2 (ALIKE)



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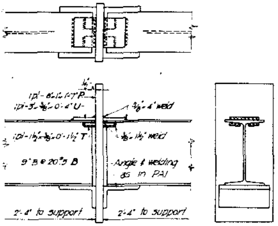

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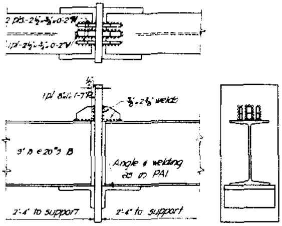

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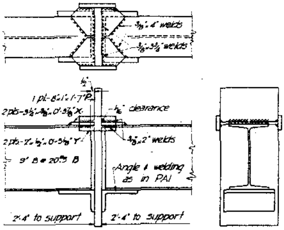


SPECIMENS PE1 & PE2 (ALIKE)

All the welding was performed by one welder, who first passed the qualification tests as laid down by the Structural Steel Welding Committee of the American Bureau of Welding. A Lincoln "Stable Arc" welder was used, operating at 20 to 25 volts and 180 to 200 amp. with $\frac{3}{16}$-in. bare Roebling wire. "Shop" welding was done with the weld horizontal, but all "field" welds were made with the beam in a position corresponding to that which it would occupy in a building. Otherwise, these operations were identical. All parts were clamped and tack welded in position before welding.

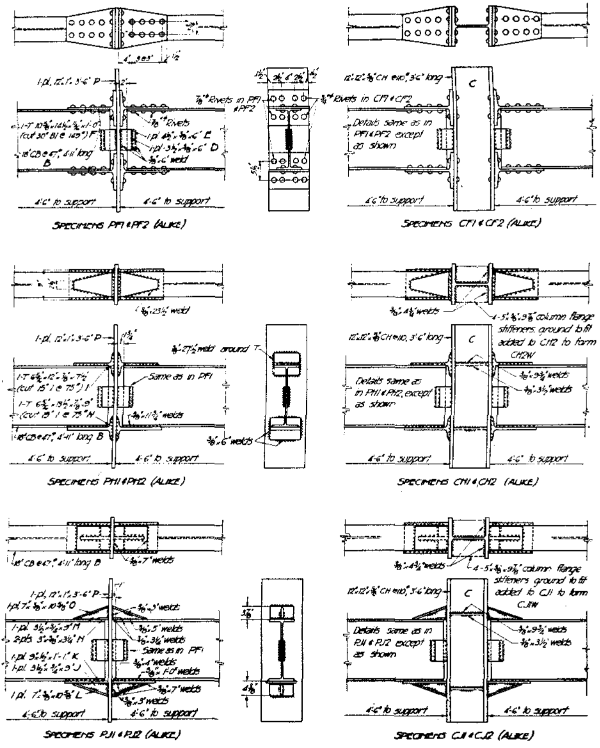


FIG. 2. *Connections of the second series.*

4. *Specimens of the Second Series*

Having established a method of procedure in the first series and desiring to extend the investigation to include wind loads, it was now thought preferable to test full-size connections. Consequently, specimens of the second series were fabricated to represent the connection of a girder (of 228 in. clear span subjected to a gravity load of 70,000 lb. or its equivalent in wind load in either direction) to the flange or web of a column six stories down from the top of a building. According to the method of design adopted, this required an 18–in., 47–lb. C.B. section for the girder. A 12 × 1–in. plate represented the web of a column, and a 12 × 12 × $\frac{5}{8}$–in. 110–lb. C.H. section represented the flange-connected column.

One type of riveted connection *F*, and two types of welded connections, *H* and *J*, were designed, as indicated in Fig. 2.

Six beam specimens, *BF*1, *BF*2, *BH*1, *BH*2, *BJ*1 and *BJ*2, for purposes of comparison, with six plate specimens *PF*1, *PF*2, *PH*1, *PH*2, *PJ*1 and *PJ*2, representing the connection of beams to the web of a column, and six "column" specimens *CF*1, *CF*2, *CH*1, *CH*2, *CJ*1 and *CJ*2 representing the connection of beams to the flanges of a column, and incorporating duplicate pairs of the various connections, were fabricated, as indicated in Fig. 3, and tested as described below. A later modification of specimens *CH*2 and *CJ*1 by the addition of stiffeners welded between the flanges of the column as shown in Fig. 2 provided the two sub-specimens *CH*2*W* and *CJ*1*W*.

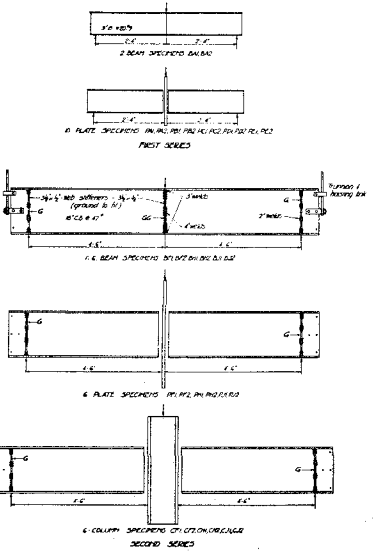


FIG. 3. *Specimens of the first and second series (connections omitted).*

In the design of these specimens the critical section of the beam was considered to exist at a distance of 10 in. from the face of the plate or column, on account of the stiffening effect of the connection, and consequently the beam was designed to resist the moment at this section while the connection plates, angles, welds, and rivets were designed to resist the moment at the support.

A nominal factor of safety of between 2.25 and 2.50 was used in the design of rivets and welds in the moment-resisting part of the connections, based on the assumed ultimate strength values given in Table I, while in other respects the permissible stresses of the specifications of the American Institute of Steel Construction were employed. Selection of the working stresses was made with a view to rendering failure of the connection welds or rivets more likely than failure elsewhere. A factor of safety of between 2.25 and 2.50 is, moreover, consistent with that obtained when wind stresses are allowed, as is occasionally the case, to exceed the stresses permitted for dead and live load by as much as 50%.

TABLE I

ULTIMATE STRENGTH VALUES OF TENSION RIVETS EMPLOYED IN DESIGN

Grip, in.	Rivet values, in lb. each	
	$\frac{3}{4}$ in. rivet	$\frac{7}{8}$ in. rivet
$2\frac{1}{2}$	30,000	35,000
$3\frac{3}{8}$	25,000	31,000

NOTE.—*Rivets, in pure tension, according to University of Toronto tests. Rivets, in single shear, based on area of the hole—48,000 lb. per sq. in. Welds, $\frac{3}{8}$ in. fillet—10,000 lb. per lin. in.*

In order that the angular rotation of the connections at the face of the support might not be unduly influenced by vertical shear deformation, or shear failure precede moment failure, two shear plates were welded on the web of each beam for all specimens, with a factor of safety of 3.43 in the welds. These shear connections were subjected to tension whenever the deformation of the tension connection greatly exceeded that of the compression connection, as in the riveted specimens, and consequently failed prematurely.

The riveted T-connection, *F*, was of a type in common use at the time of fabrication of the specimens. Although the riveting arrangement adopted for the connection to the face of the column would, where the attachment was to an unstayed flange, give higher column deformation than if the rivets had been grouped closer to the column web, this particular source of flexibility did not arise where a central plate, rather than a column section, separated the two cantilevers. The use of $\frac{3}{4}$-in. rivets through the flanges of the column and of $\frac{7}{8}$-in. rivets through the 1-in. plates of the plate specimens was in conformity with the expected strength of rivets of different grips in tension, as indicated by tests made some years ago at the University of Toronto (1).

In the design of the welded T-connection, *H*, a split I-section with a heavy web and narrow, heavy flanges was found desirable, in order to keep the tensile stresses in the tapered web of the upper T within safe limits and to minimize the deflection of the flanges of both T's.

The gusset-plate type of welded connection, *J*, was so proportioned that the long median line of each gusset when produced intersected the neutral axis of the specimen at the load point, 54 in. out from the back of the connection. Care was taken to ensure adequacy of the plates as well as of the welds of the connection.

The beams for the specimens were sawn from three 60-ft. lengths. These had been ordered from the mills and were required to be taken from the same rolling. The welding was performed by the man who did the welding for the first series. A Lincoln "Stable Arc" welder was used with "Stelco Electrod," medium carbon, No. 4, $\frac{3}{16}$ in. diameter, manufactured by the Steel Company of Canada. This is a "bare wire" electrode and complied with the American

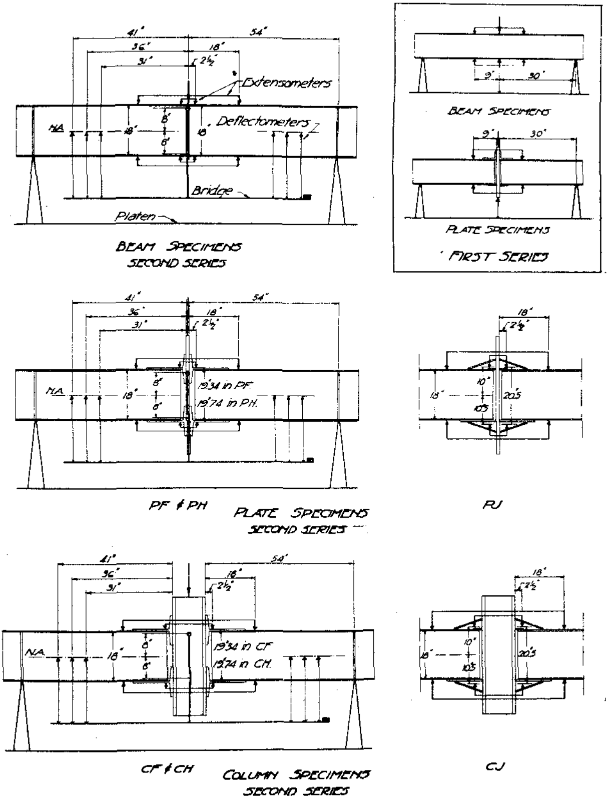


FIG. 4. *Location of gauge points for extensometers and deflectometers.*

Welding Society Specification EIA. Current specified for horizontal welds was 225 amp. and for vertical welds 180 amp.

5. *Instruments*

Two types of instruments were used to measure the deformation of the specimens, *(a)* extensometers, with accessories, to measure horizontal deformation between points on or near the flanges of the beam, and *(b)* (second series only) deflectometers, with accessories, to measure the vertical movements of points on the neutral axis of the beam.

(a) Extensometers and Accessories. In the first series only one gauge length was used for the two extensometers, the gauge points being symmetrically located on the centres of the flanges beyond the ends of the connections. In the second series it was considered desirable to be able to analyze and allocate portions of the over-all deformation as obtained in this first series and for this reason a second pair of extensometers was used on intermediate gauge points. The locations of the gauge points used in each series are shown in Fig. 4.

These gauge points, being on opposite sides of the plate or column, are not directly inter-accessible, and consequently special extensometers had to be constructed. Two transverse square tubes carrying the contact points, multiplying lever and Ames dial were clamped by means of split sleeves to two longitudinal round tubes of suitable length. The instrument and the method of its attachment to the specimen are shown in Fig. 5.

In the specimens *PJ* and *CJ* (second series) the gauge points for the shorter extensometers were located on the vertical gusset plate of the connection, and special girdles attached to these points were required to convey their movement to the extensometers, as shown in Fig. 5. A correction had to be applied to the readings obtained with the girdles to determine the true movement of the gauge points on the gusset plates.

The Ames dial, combined with the multiplying lever, indicated a movement of the gauge points of 1/5000 in. per division. Tenths of a division, or fifty-thousandths of an inch, were estimated and recorded. The extensometers were periodically calibrated on a special micrometer gauge.

(b) Deflectometers and Accessories. Simple deflection measurements between the bottom of the specimen and the platen of the testing machine were taken in the first series but proved useless on account of errors due to the settling at the knife edges and deformation of the platen. A larger testing machine was used with the second series with a special form of laterally rocking knife edge, but similar difficulties were encountered. As much as 25% of the deflection indicated was found to be due to deformation outside of the specimen. To eliminate this error, and to establish a datum plane fixed in inclination with respect to the axis of the column and in height with respect to its centre, it was decided to measure the vertical movement of points on the neutral axis of the beams as related to a bridge hung from, and aligned at right angles to, the central vertical element of the specimen. The bridge and its method

of suspension, and the yokes carrying the Ames dials and their attachments to the neutral axis are shown in Fig. 5. The particular advantage of this method of measurement in the present investigation was that it gave the deformation of each half of the specimen, with respect to the central vertical

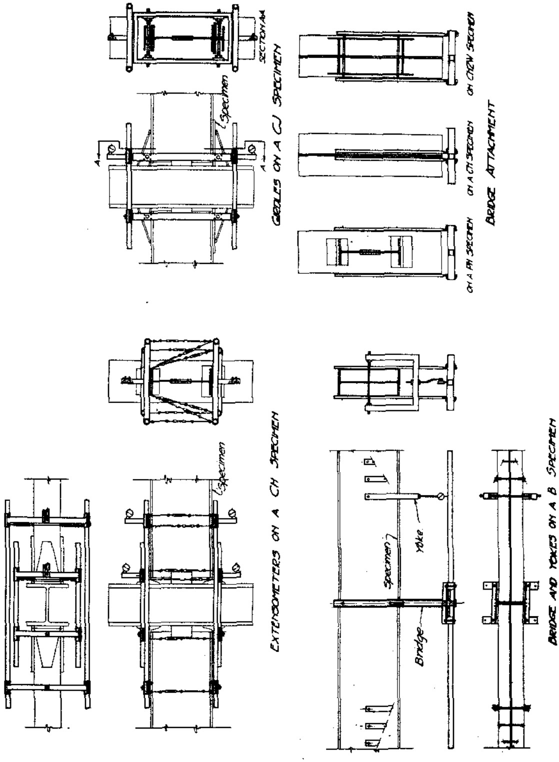


FIG. 5. *Attachment of instruments employed in measuring deformations.*

section, separately, and, in the case of the connected specimens, indicated the relative rigidity of the two connected cantilevers.

The Ames dials indicated 1/1000 in. per division. Tenths of a division, or ten-thousandths of an inch, were estimated and recorded.

The instruments assembled on a specimen of the second series are shown in Fig. 6.

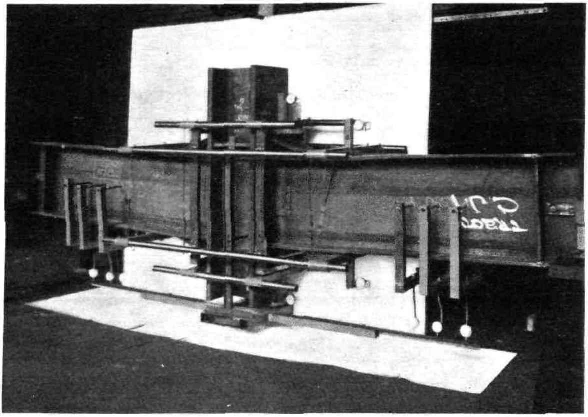


FIG. 6. *Instruments assembled on specimen CJ1W (second series).*

6. *Test Procedure—First Series*

Each specimen of the first series was tested in an inverted position in a 100-ton Riehlé machine. The supporting knife edges might thus be considered as applying the load to the two cantilever arms and the loading knife edge as taking the reaction at the centre of the specimen. Bearing plates were used to distribute the load or reaction where it was applied to the beam flange.

One extensometer was attached to each flange, and one deflectometer was supported on the platen of the machine, as indicated in Fig. 4.

The first of each pair of specimens was subjected to three loads, the first and second, by 2000-lb. increments, to approximately 26,000 lb., and the third by one 1000-lb. increment and then by 2000-lb. increments to 27,000 lb., followed by 1000-lb. increments to failure.

When these were completed the second specimen of each pair was loaded by 2000-lb. increments to 30,000 lb. and then by 1000-lb. increments to failure.

The results obtained for one beam, and one plate specimen, for all loads applied, are shown in Fig. 7.

The large initial set produced by the first load on welded specimens is well illustrated by the curves for the tension flange of *PA*1. This effect combined

with increasing stiffening due to the improving seating of the compression connection is shown by the curves for the compression flange. The net result combined with settling at the knife edges and undetermined deformation of the platen is shown by the deflection curves. The marked difference between beam and plate specimens in all cases is indicative of the imperfection of the restraint developed by the connection. Finally, while failure actually occurred in the tension connection, it can be seen that for safe loads it provided the greater rigidity.

In the simulated beam the initial set produced by the first load involves a permanent loss of restraint, and therefore in the study of restraint, as distinct from wind bracing rigidity, it is necessary to use the curves for the initial loads, extending them, as indicated by the dotted line, to link up with the latter portion of the failure load.

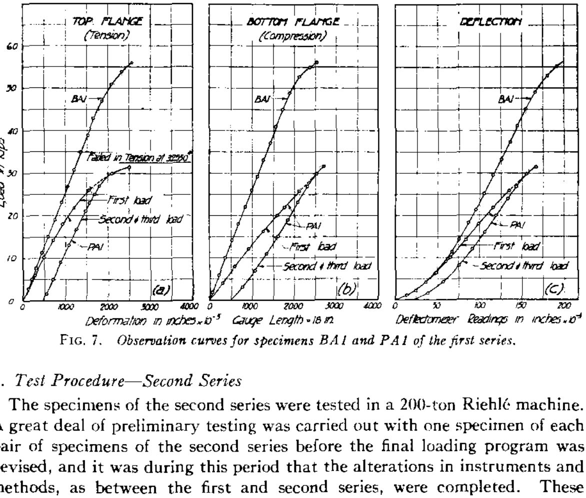


FIG. 7. *Observation curves for specimens BA1 and PA1 of the first series.*

7. *Test Procedure—Second Series*

The specimens of the second series were tested in a 200-ton Riehlé machine. A great deal of preliminary testing was carried out with one specimen of each pair of specimens of the second series before the final loading program was devised, and it was during this period that the alterations in instruments and methods, as between the first and second series, were completed. These included: (i) the introduction of the second pair of extensometers to differentiate between connection to beam and connection to column, involving the use of girdles in the *J* specimens; (ii) the radical change in the method of measuring deflection involving the suspended bridge and deflectometer yokes previously described; and (iii) the study of the behavior of the specimens under repeated and reversed loads, applied and released, and the evolution of a loading program that would yield information applicable to the two-fold problem of beam continuity and wind bracing rigidity.

As has been pointed out in connection with the results obtained in the first series, only one loading is required to determine the degree of restraint or continuity developed by a connection. A wind bracing connection, however, may be subjected to complete reversal of stress which may involve both elastic and non-elastic slip, and further, it has been shown that initial set occurring during the first load alters the behavior of the specimen during subsequent loads. Consequently, to determine the final rigidity of a wind connection, an adequate series of loads must be applied to eliminate initial set, and reveal the full value of slip due to reversal of load, as well as the normal elastic deformation of the specimen.

To fulfil these requirements each specimen was subjected to five loads applied and released to 5000-lb. or 10,000-lb. stages from 0 to 75,000 lb. and a sixth load to failure. The first two loads, termed "normal loads," were applied so that the connection was stressed as by a gravity load in the simulated beam. The third and fourth, termed "reversed loads," were applied in the opposite direction, while the last two loads were again normal loads. Readings on the four extensometers and six deflectometers were made after each stage in the application and release of each load.

Important improvements were devised in the method of applying and releasing the loads on the specimens of the second series. A potentiometer type of controller on the motor actuating the loading screws enabled the operator to balance the beam of the machine at any predetermined load with great accuracy and speed.

Handling of these specimens was facilitated by the use of a traveling hoisting tackle and trunnions attached to the ends of the specimens. By this means the specimen could be rotated about its horizontal axis, for the purpose of reversing the load, without removing the extensometers. It was obviously necessary, however, to remove the bridge and deflectometer yokes at each reversal.

The loads applied to the specimen were numbered consecutively throughout the history of the specimen and involved the application, "ON," and release, "OFF," of the load; and certain significant load applications or releases or combinations thereof were lettered for convenient identification, such as the application of the initial normal load, *A*, the release of a repeated reversed load (following a normal load), *X*, etc.

As this loading program was adopted after considerable testing had been carried out, and, further, as premature partial failure occurred in one case (*CH2*) necessitating the inversion of *CH2W*, certain variations of the standard program will be found in the record of tests performed.

Typical observation curves are given in Fig. 8 to illustrate the characteristic differences in observed deformation due to load sequence, type of specimen and type of measurement. All curves shown are for previously unused specimens; curves obtained with used and unused specimens of the same type will be seen in Fig. 9 and discussed later.

The existence and evaluation of set and slip are well illustrated in Fig. 8. In the beam and welded specimens, *(a)* to *(e)*, where no slip occurs the amount of initial and subsequent set is clearly depicted. The amount of initial set depends on the stress and proportional limit in the part of the specimen

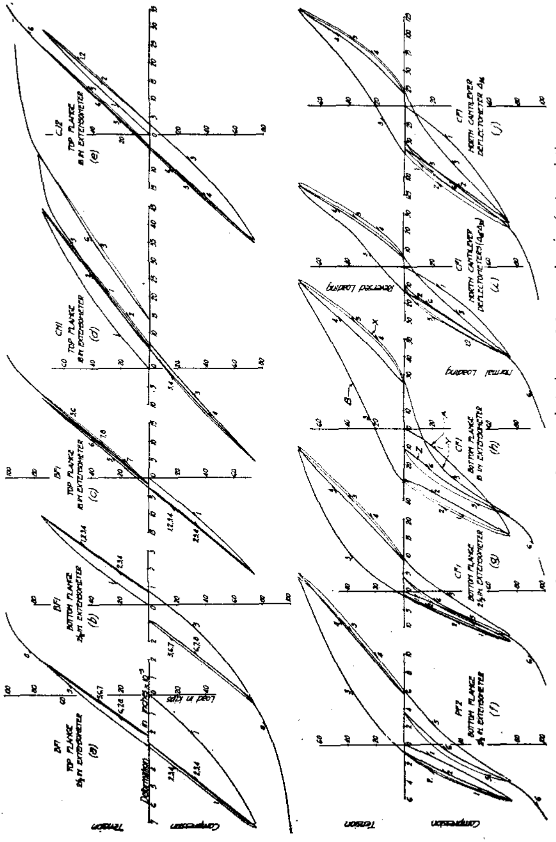


FIG. 8. *Typical observation curves for unused specimens of the second series (note variation in scales of deformation).*

considered and on the order of loading. It will be seen from *(a)* and *(b)* that initial set occurs in the central 5-in. section of the beam to a marked degree during the initial application of both tension and compression; also that it is greater in compression than in tension and that either is slightly decreased by following the other. Similar results were obtained for all the "beam" specimens. It is thought, however, that the introduction of cast metal in the welding of the web stiffeners in this section is largely responsible for the low limit of proportionality and the correspondingly great amount of initial set.

A comparison of *(a)* and *(c)* (note difference in scale) will show that there is very little additional set in the region between the 2½-in. and 18-in. gauge points.

By comparing *(c)* with *(d)* and *(e)*, and considering the effect of load sequence previously discussed, certain important differences will be noted: first, that greater initial set occurred in the welded specimens than in the beam specimens; second, that the larger initial set occurred in tension in the *H* specimen *(d)* and in compression in the *J* specimen *(e)*; and, third, that considerable subsequent set developed in the top (tension) flange of *CH*1 during the application of the fifth load. This was evident to some degree in all the *H* specimens and was due to the severe unequal stress distribution in the welds caused by the bending, in the *PH* specimens, of the flanges of the connecting T, and in the *CH* specimens, primarily, of the flanges of the column.

Observation curves are given for the riveted specimens, *(f)* to *(j)*, for the three types of measurements made. In comparing these it must be remembered that while *(f)*, *(g)* and *(h)* (extensometers on the bottom flange) indicate the horizontal deformation of part or all of *two* bottom attachments, both in compression (or tension), *(i)* and *(j)* (deflectometers) indicate the difference in deflections at 31 in. and 41 in., or slope, and deflection at 36 in., of one cantilever comprising one bottom and one top attachment, the one in compression and the other in tension for the normal load, and, *vice versa*, for the reversed load.

As will be seen from Fig. 9 and other observational curves not here reproduced, the difference in behavior of the two flanges when similarly stressed is negligible, but the difference between the behavior of a flange in tension and compression is quite appreciable. This is evident from the similarity of the deflectometer curves for normal and reversed loading and the marked difference between the extensometer curves for tension and compression.

An analysis of the results obtained with, say, the 18-in. extensometer on the bottom flange of *CF*1, Fig. 8 *(h)*, during the standard loading program will indicate the significance of the various loads applied and will justify the use of the particular loads chosen to represent the behavior of the connection.

Load 1 is a "normal" load. It produces initial compression in the bottom flange and the resulting deformation includes initial set in compression and, as will be obvious from results obtained with subsequent loads, a certain

amount of elastic and non-elastic slip, as well as proportional deformation. This is termed the *A* load. On the release of this load the elastic deformation only is recovered. Load 2 is a repeated normal load and includes elastic slip, subsequent set if any, and proportional deformation. Load 3 is the first reversed load. It produces initial tension in the flange and includes initial set in tension, elastic and non-elastic slip and proportional deformation. This is termed the *B* load. On the release of this load elastic deformation only is recovered and, therefore, the net deformation for Load 3 includes non-elastic slip and initial set in tension. Load 4 is a repeated reversed load and includes elastic slip, subsequent set, if any, and proportional deformation. On the release of this load, termed the *X* load, elastic slip and proportional deformation are recovered. Load 5 is the first normal load of the second cycle and includes non-elastic and elastic slip, proportional deformation, and subsequent set, if any. This is termed the *Y* load. On the release of Load 5, elastic slip and proportional deformation are recovered, and, therefore, the net deformation for Load 5 includes non-elastic slip and subsequent set in compression, if any. Load 6 is the second normal load of the second cycle and includes proportional deformation, elastic slip and subsequent set in compression, and is carried to failure. This is termed the *Z* load.

The comparatively large loops obtained in the curves for the riveted specimens are due mainly to friction between the slipping parts, but their existence in the curves for the other specimens would indicate that hysteresis (see *(e)* Load 2) and instrumental lag (see *(b)*, Loads 6 and 7) were present to a small degree.

Subsequent set occurred to some extent in the top flanges of the *F* and *H* specimens when in tension, due to uneven stress distribution in the rivets or welds and the low factor of safety adopted for these parts in design, but elsewhere it was negligible.

Based on the foregoing observations the following significant loads were used for the purposes indicated:—

(1) To establish the value of the connection, or part, in resisting wind loads, alternately reversed, or repeated.

(a) Alternately Reversed Wind Loads. The performance of the specimen during the release of the fourth load and the application of the fifth load, termed the *XY* load, was used to represent the steady state behavior of the flange or cantilever when subjected to a complete reversal of load. The resulting deformation includes proportional deformation and elastic slip in compression and tension, non-elastic slip, and subsequent set in one direction, if any, but excludes initial set in both directions.

(b) Repeated Wind Loads in One Direction. The performance of the specimen during the application of the sixth load, termed the *Z* load, was used to represent its behavior when subjected to repeated loads in one direction and carried to failure. The resulting deformation includes proportional deformation and elastic slip and subsequent set in one direction, but excludes initial

set in both directions and non-elastic slip. Within their load ranges, the second and fourth loads would serve the same purpose except for the decrease in slip friction with successive loads and the consequent gradual change in the value of elastic slip.

(2) To establish the value of the connection or part in developing restraint or beam continuity.

(a) Initial Restraint. The performance of the specimen during the application of the first load combined with its subsequent performance during the application of the higher stages of the sixth load, termed the AZ load, was used to represent its behavior when subjected to an initial load carried to failure. The resulting deformation includes proportional deformation, partial elastic and non-elastic slip, initial and final set.

(b) Subsequent Restraint. The net deformation of the specimen during the first five loads combined with its performance during the application of the sixth load, termed the Z' load, was used to represent its behavior when subjected to repeated loads finally carried to failure.

The method of abstracting the mean XY and Z curves from the original observation curves is illustrated in Fig. 9 for the 18-in. extensometers on the two PF specimens. These curves are plotted assuming reversed load to be negative and normal load positive; consequently tension and compression will be positive and negative respectively for a top flange and negative and positive respectively for a bottom flange. Complete observation curves are given for the four flanges in *(a)*, *(b)*, *(c)* and *(d)*. XY curves are isolated in *(e)* and *(f)* and the Z curves in *(g)* and *(h)*. The mean curves for each flange and the final mean for the two flanges are shown in *(i)* and *(j)*.

Deformations indicated in the observation curves for a given flange include the deformations of the two attachments to that flange as indicated by the extensometer. The mean curve for the two top flanges therefore includes half the sum of the deformation of the four top attachments, and likewise the mean curve for the bottom flanges includes half the sum of the four bottom attachments. The final mean of these two means consequently includes one-quarter of the sum of the deformation of the eight attachments and represents the combined deformation of the mean top attachment and the mean bottom attachment; that is, it represents the mean deformation of the four cantilevers involved.

The deformations are given in inches as determined by the extensometers and a computed scale in radians is added to indicate the rotation of the section due to the horizontal deformations in the flanges, allowing for the vertical distance between top and bottom flanges. These scales refer to two top attachments, two bottom attachments, or, in the case of the final mean, one top plus one bottom attachment, that is, one complete connection.

The XYZ loop, Fig. 9 *(k)*, is a combination of the final mean curves within the limits $\pm$ 70,000 lb. and therefore represents the rotation of the section, 18 in. from the support, during repeated complete reversals of load in the

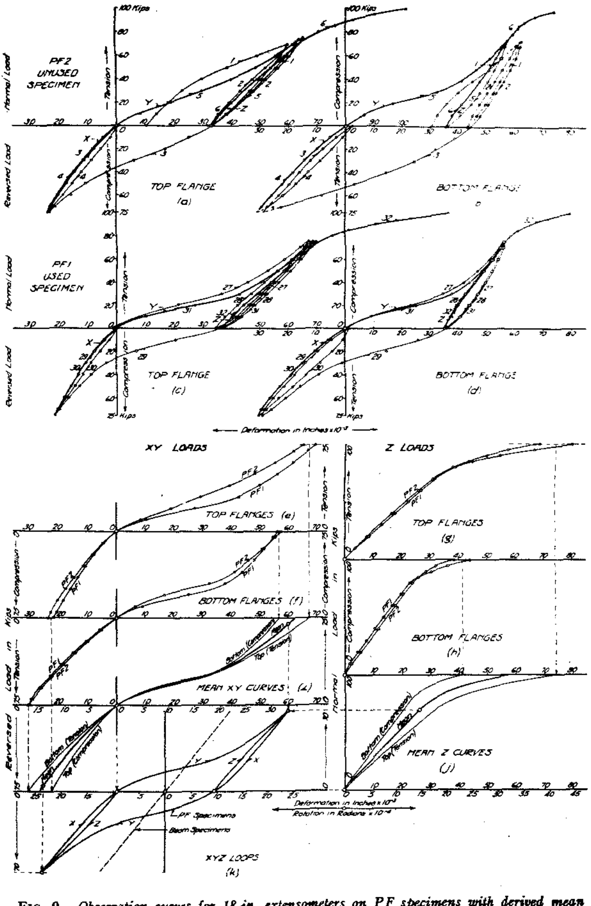


Fig. 9. *Observation curves for 18-in. extensometers on PF specimens with derived mean curves and XYZ loops.*

XYXY loop, and during repeated loads in the same direction in the *XZ* loop. The corresponding "loop" for the mean beam specimen is represented by the dotted line, the width of the loops being imperceptible at this scale.

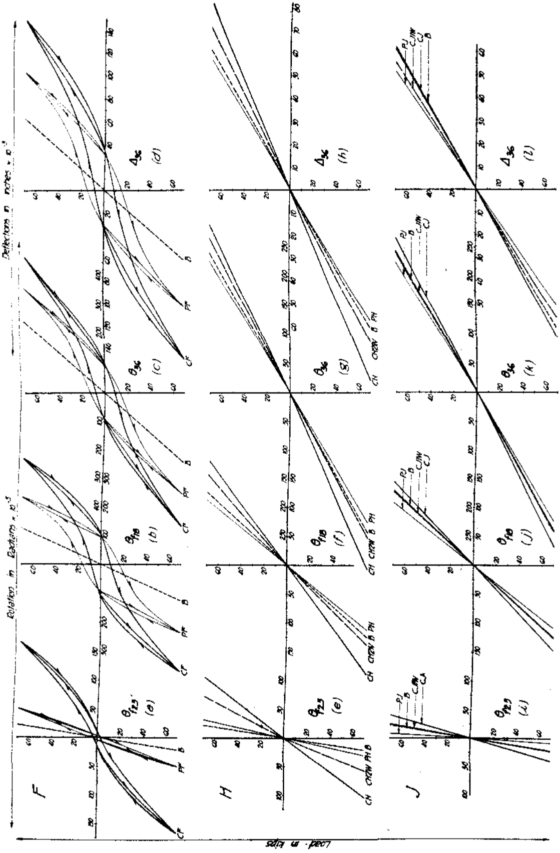


FIG. 10. *XYZ loops for all specimens of the second series (note variation in scales of deformation).*

Similar mean curves for the XY and Z loads were obtained for the 2½-in. extensometers, the difference between the 31-in. and 41-in. deflectometers, and for the 36-in. deflectometers. The work was done graphically on a large scale and when the mean curves were obtained appropriate scales were used to measure the curves and tabulate the deformation in inches or radians as required. These tables indicate the mean performance of the specimens tested and form the basis of subsequent analysis. They are represented graphically in Fig. 10 in the form of XYZ loops for all types of specimens and all measurements.

The XYZ loops in Fig. 10 are grouped for purposes of comparison as follows:—

In each row are all the loops for one type of connection, F, H or J.

In each column are all the loops for one type of measurement (see Fig. 4). The symbols $\theta_{f2\cdot5}$ and θ_{f18} are used to represent the flexural slope at sections 2½ and 18 in., respectively, from the supports (*i.e.* from the centre of the beam or face of the plate or column), as determined by the "2½-in." and "18-in." extensometers. The deformation is indicated in radians. θ_{36} represents the slope, produced by flexure and shear, at a section 36 in. from the support as determined by the difference in deflection between points 31 in. and 41 in. from the support. Again, the deformation is indicated in radians. $\triangle_{36}$ represents the deflection of a point on the neutral axis of the cantilever 36 in. from the support, this deformation being indicated in inches. In each group are the loops for the mean beam, plate, column and modified column specimens.

The scale for plotting radians is uniform except in *(b)* and *(c)*, which are plotted to half the scale used elsewhere. The scale for plotting deflection was chosen so that θ_{36} and $\triangle_{36}$ for the beam are equal for each type of specimen; consequently the scale used in *(d)* is half that used in *(h)* and *(l)*.

The XYZ loop for θ_{f18} of the CF specimen is reproduced in Fig. 11 *(a)* to illustrate the various forms of deformation that occur in the riveted specimens to which reference has already been made. The significance of Fig. 11 *(b)* is discussed in Art. 25.

Proportional deformation of a perfect specimen according to Hooke's law is indicated approximately by the tangent to the centre line of the XZ loop.

Slip may be defined as the relative movement of connected parts; it may be tangential, as between the web of the T's and flanges of the connected beam, in the F specimens, caused by deformation or movement of the connecting rivets, or it may be perpendicular to the connected surfaces, as between the flanges of the T's and columns, caused by bending of these flanges resulting in improved bearing in compression. The former type is largely non-elastic, that is, it is not recovered on release of load; the latter, as might be expected, is nearly all elastic.

Elastic slip is indicated in the XYZ loops by the curvature of the XZ loop; its full value is represented by the distance along the X-axis between the proportional deformation tangent and the XZ loop.

Non-elastic slip is indicated by the distance between the *XZ* loops for loads in opposite directions.

Initial set, as has been shown, does not occur during the loads represented by the *XYZ* loop but is indicated in the observation curves of Figs. 8 and 9 by the difference between the deformations that occur during the first and second loads.

Proportional deformation is the only type of deformation that occurs to any appreciable extent in the beam and welded specimens; hence the "loops" for these specimens, *B*, *H* and *J*, become practically straight lines.

With these interpretations in mind and recollecting the location and nature of the measurements made, a brief study of the *XYZ* loops in Fig. 10 will serve to indicate the characteristic differences in the behavior of the connections tested.

In the $\theta_{f2.5}$ loops, *(a) (e)* and *(i)*, Fig. 10, the difference between the *B* and *P* loops represents the increase in deformation due to 2½ in. of the connection adjoining the plate, including the rivets in the *F* specimens; the difference between the *P* and *C* loops indicates the deformation in the column and any effect it may have on the deformation of this part of the connection; the difference between the *C* and *CW* loops shows the effect of the column flange stiffeners in reducing the deformation of the column. The relative rigidities of this part of the connection for different types, the effect of the type of connection on the deformation of the column, and the effect of welded column flange stiffeners are clearly shown.

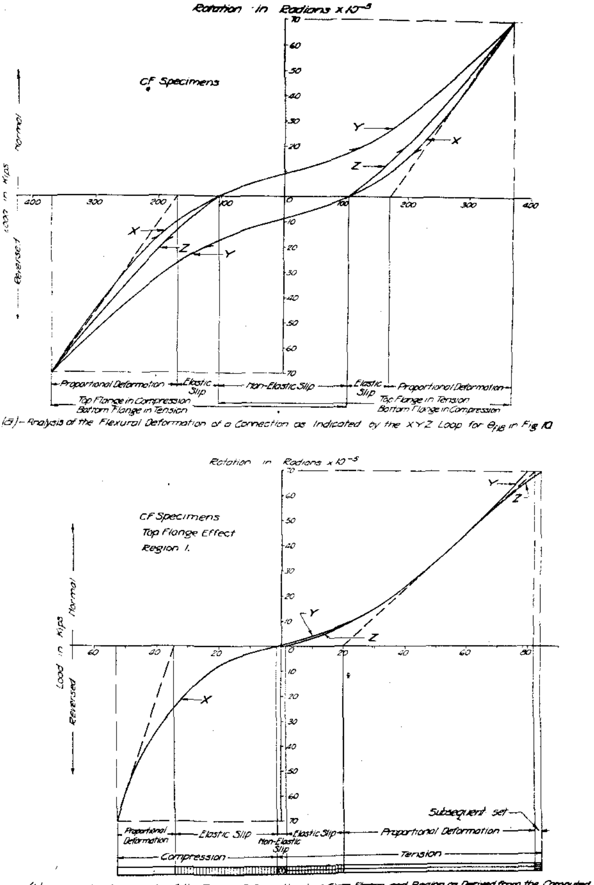
The θ_{f18} loops, *(b)*, *(f)* and *(j)*, include the deformations represented in the previous loops and the differences between *B*, *P*, *C* and *CW* are similarly significant; consequently only the increase or decrease in these differences between $\theta_{f2.5}$ and θ_{f18} indicates the effect of the connection beyond the 2½-in. gauge points.

In the *F* specimens the increase in non-elastic slip should be noted. A slight decrease in proportional deformation occurs in *F* and *H*, owing to the stiffening effect of this part of the connection on the beam flange; it is indicated by the decrease in the difference of slope between the beam and the *P* or *C* loops.

These loops, $\theta_{f2.5}$ and θ_{f18}, are derived from extensometer measurements. The remaining loops, θ_{36} and Δ_{36}, are derived from deflectometer measurements and serve to confirm and extend the information obtained from the extensometer measurements, which do not indicate the existence of vertical shear, but are essential for the analysis of flexural slope development.

The only difference between θ_{f18} and θ_{36} is the increase in flexural slope in this section of the beam and the addition of the shear slope at this point, both of which are common to all specimens, and are indicated as part of the beam deformation. The differences, therefore, between *B*, *P* and *C* should be the same in θ_{36} as in θ_{f18} if all the beam sections used are identical.

The Δ_{36} loops, *(d)*, *(h)* and *(l)*, are plotted to a scale that produces a slope in the beam loop equal to that of θ_{36} for the beam. Since the differences



(a)– Analysis of the Flexural Deformation of a Connection as Indicated by the XYZ Loop for θ_{HB} in Fig. 10

(b)– Approximate Analysis of the Flexural Deformation in a Given Flange and Region as Derived from the Computed Curves in Figs. 19 and 20, and Indicated in Fig. 17.

FIG. 11. *Analysis of flexural deformation.*

in flexural slope between the beam and the other specimens are developed near the plate or column, the differences in deflection for these specimens will be approximately proportional to the differences in flexural slope and, therefore, since flexural slope and flexural deflection for the beam are made equal, any change in these differences between θ_{36} loops and Δ_{36} loops will indicate roughly the change in shear deflection due to the connection. On this basis an increase in shear deflection is indicated for *F* and *H* specimens and a decrease for *J* specimens.

III. Theoretical Considerations

8. *General*

So complicated in its behavior is even the simplest welded connection, and so little is known of the elastic properties of welds laid down under varying conditions, that determination by theoretical processes alone of the degree of restraint attainable by the use of a given connection is impracticable. Experimental means had to be devised to obtain this information.

9. *Relation of Specimens of First Series to Simulated Beam*

As has been pointed out in Section II, double-cantilever specimens were devised to simulate a uniformly loaded floor beam more or less restrained at the ends. Calculation showed that for the specimens of the first series a double cantilever having a clear span of 28 in. on either side of a centre support, as shown in Fig. 12 *(a)*, would, if loaded at each end by a 12,000-lb.

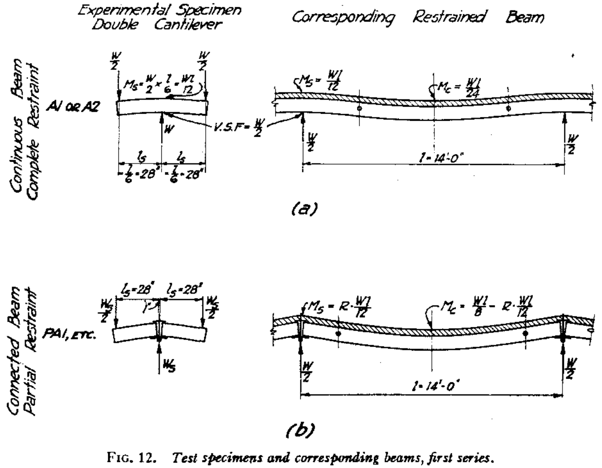


FIG. 12. *Test specimens and corresponding beams, first series.*

load, $\frac{1}{2}$ W, give for each span exactly the same shear and moment at the support as would exist at the supports of the assumed 14-ft., perfectly restrained, uniformly loaded beam. The deflection would nowhere in the 28-in. cantilever span exceed the deflection in the end 28 in. of the 14-ft. beam by more than 3%, in spite of the fact that while the load on the simulated beam is uniformly distributed, the load on the double cantilever is concentrated. Obviously, then, the comparatively short welded connections would, in the test specimens, be subjected to a shear and moment relation at the face of the support exactly equal to that in the simulated, perfectly restrained, beam and to a deflection within the length of the connection almost exactly so. Actually, of course, a specimen designed to give the same moment and shear at the cantilever supports as at the ends of a perfectly restrained beam would not exactly represent the simulated beam if the restraint for the latter became less than perfect. Less load on the standard double-cantilever specimen described above would give the same end moments for the cantilevers as would be given at the ends of a partially restrained beam carrying a total load of 24,000 lb. This arises from the fact that, as the joint yields, the end moment becomes relatively smaller with respect to the total moment in the span, and consequently a representative cantilever specimen would need to be loaded more lightly than if the restraint in the simulated beam were perfect.

It thus happens that the shear in the double-cantilever specimen, designed as above, is less than the shear at the ends of the simulated beam. To give the same end shear for the two, as well as the same moment, the span of each cantilever arm in the specimen should be, as established by Equation (15) of this Section, $l_s = \frac{l}{6} \cdot \frac{RW}{W_s}$, in which l is the span of the simulated beam, W the load on it, W_s the load on the cantilever specimen and R the coefficient of restraint, that is, the ratio between the existing end moment and the end moment if the beam were perfectly restrained.

Since the degree of restraint is not known beforehand, it is not possible to fix the appropriate cantilever span in order that the end shears may be exactly the same for the cantilever specimen and the simulated beam at the time that the moments are equal. Moreover, the cantilever span would theoretically need to vary with each load stage. Consequently, for the first series, a standard effective span for each of the two cantilevers of a specimen was made $\frac{1}{6}$ of the span of the simulated partially restrained beam, as indicated in Fig. 12 *(b)*. Hence, while the cantilever specimens could be loaded to represent any end moment in the simulated beam, the simultaneous shear would be somewhat less than the end shear in the simulated beam. Where the coefficient of restraint is not far from unity this difference of shear is of no particular consequence.

10. *Relation of Specimens of Second Series to Simulated Beam*

The specimens of the second series were designed, as has been indicated, to investigate wind bracing rigidity as well as beam continuity and therefore

in the design of these specimens a compromise was made between the moment-shear relations existing in the two problems. It was also deemed desirable to assume that the connection increased the stiffness of the beam within the length of the attachment, approximately 10 in., and therefore the critical section of the beam occurred at a point 10 in. from the face of the column. The beam was designed as a continuous beam with a span of 228 in., and hence the corresponding specimens, according to the previous article, should have cantilever spans of 44 in. each. For the reasons given above, however, the connection was designed to develop the strength of this beam at a point 10 in. from the support and therefore a cantilever span of 54 in. was chosen. This departure from the ideal ratio, $\frac{l}{l_s} = 6$, involves a decrease in the shear-moment ratio, but the effect of this change in shear on flexural slope is assumed negligible, and since flexural slope is determined by measurements unaffected by shear deflection, the results obtained with these specimens and interpreted by the appropriate equation derived in Art. 23 accurately represent the performance of the simulated beam in the development of restraint under gravity load.

The ideal cantilever span required to simulate a beam subjected to wind load is $\frac{l}{2}$, assuming the point of inflection to occur at mid-span. As this span could not be accommodated in the testing machine it would be necessary to apply corrections to the results obtained on any specimen to determine the performance of the simulated beam subjected to wind load and, therefore, the cantilever span chosen to produce design stresses simultaneously in the beam and connection as indicated above was considered satisfactory.

Consequently, double-cantilever specimens were fabricated for the second series with each cantilever arm measuring 54 in. from the back of the connection, or the face of the cantilever support. The critical section in the beam was considered as arising at 10 in. from the face of this support, so that while for the connection itself the maximum moment was assumed to exist at the face of the cantilever support, the moment employed in the proportioning of the beam was assumed to occur at 10 in. from the back of the connection. Under the design load of 70,000 lb. on each double-cantilever specimen the design moment for a connection was, therefore, 35,000 × 54 = 1,890,000 in.-lb. and the design moment for the beam section employed in the specimen was 35,000 × 44 = 1,540,000 in.-lb.

Owing to the effect of varying coefficient of restraint the shear in the test specimen was not exactly the same as that which would exist in the simulated, partially restrained, beam under equal end moments. However, as the coefficient of restraint was fairly high under loads approximating the working loads, this disparity in the shears is not believed to have affected the behavior of the specimens appreciably.

11. *Slope Angle Related to Extensometer Measurements*

The general basis for rating the rigidity of a connection was to compare the flexural slope developed in the connected beam at any point beyond the connection with the flexural slope at the same point in a uniform uncut beam similarly loaded. Cut and connected double-cantilever specimens were consequently tested in direct comparison with uncut and unreinforced specimens.

As has been described in Section II, the primary method of determining the angular change was to attach to each flange a special extensometer which would indicate the change in length of a gauged distance symmetrically placed about mid-span of the specimen. The difference, for example, in extension of the tension flange of the specimen and the tension flange of the plain beam would obviously be due to the yield of the connection itself, modified by the reinforcing effect of the connection material on the beam flange.

Flange length changes shown by the extensometers were utilized to determine, by the method described below, the equivalent end slope angle due to flexure for a complete connection.

Let the original gauge length on the bottom, normally the compression, flange of a specimen be l_b and that on the top, or tension, flange be l_t as shown in Fig. 13. Under load, l_b becomes $l_b - \delta l_b$ and l_t becomes $l_t + \delta l_t$. The relative horizontal movement of two opposite gauge points on one side of the centre, employing the average for the two sections, is

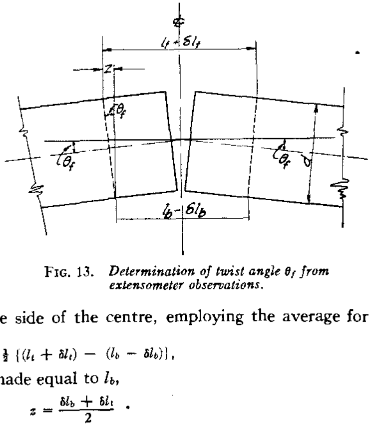


FIG. 13. *Determination of twist angle θ_f from extensometer observations.*

$$z = \tfrac{1}{2}\{(l_t + \delta l_t) - (l_b - \delta l_b)\},$$

and since l_t is conveniently made equal to l_b,

$$z = \frac{\delta l_b + \delta l_t}{2}.$$

If d be the beam depth, then the average inclination or twist of the section through opposite gauge points due to flexure, with respect to its original direction, is

$$\theta_f = \frac{z}{d} = \frac{\delta l_b + \delta l_t}{2d} \qquad (1)$$

12. *Slope Angle Attributable to Connections Alone*

Of course, not all of the slope angle indicated in Equation (1) is due to the distortion of the connections, since extensometers similarly placed on plain,

uncut, perfectly restrained double cantilevers would show length changes under load. These length changes may be excluded from those for the uncut specimens by subtraction. For example, using subscript numbers 1 and 2 to indicate quantities derived from a cut and an uncut specimen respectively, the average of the slope angles for the two end connections due to flexure only would be

$$\theta_f = \frac{(\delta l_{b2} - \delta l_{b1}) + (\delta l_{t2} - \delta l_{t1})}{2d} \quad . \tag{2}$$

This angle, θ_f, is due to the yield of the connection, modified, and sometimes more than offset, by the reinforcing effect of the connection material on the beam flanges. Shear deflection in the specimen, resulting in equal vertical movements of two cross sections on opposite sides of the load point will not affect the value of θ_f as determined by flange extensometers.

Obviously the contribution of the compression (bottom) part of the connection to the slope angle is

$$\theta_{fb} = \frac{\delta l_{b2} - \delta l_{b1}}{2d} \tag{3}$$

and that of the tension (top) part is

$$\theta_{ft} = \frac{\delta l_{t2} - \delta l_{t1}}{2d} \, . \tag{4}$$

The following theoretical considerations, while generally applicable, refer in particular to specimens of the second series on which, alone, the necessary measurements were made. See Fig. 14.

13. *Determination of EI for the Mean Plain Beam*

With a view to determining the effect of the connections themselves, it was necessary to ascertain carefully the elastic properties of the plain, or uncut beams. The mean of the values of any given quantity for the six plain beams will be spoken of as the value of that quantity for the "mean plain beam."

There being two consecutive gauge points on each flange in each half of the specimen belonging to different extensometers, that is, at $x = 2.5$ and $x = 18$ in. from the middle of the specimen, EI, the product of the modulus of elasticity and the moment of inertia, may be determined for the mean plain beam by two independent series of extensometer readings. The longitudinal deformations disclosed by them when introduced in Equation (1) will yield values of the flexural slope angles developed respectively in the first 2.5 in. and in the first 18 in. of the beam from the load point. By considering also differences of the angles there are thus made available flexural deformation data for three zones, zero to 2.5, zero to 18 and 2.5 to 18 in.

Application of these angles to the determination of EI may be made by writing the usual expression for flexural deflection at any point distant u_n from the free end of a uniform cantilever of span l_s carrying a single load $\frac{1}{2} W_s$ at the free end, as shown in Fig. 15. Differentiating this with respect to u_n, the flexural slope, θ_{fn}, at the section is found in terms of EI and certain known quantities. The value of this angle having already been found by

extensometer observations, EI is readily ascertained. The mean value adopted, based on a large number of determinations, was 22,600,000,000 lb. per sq. in.

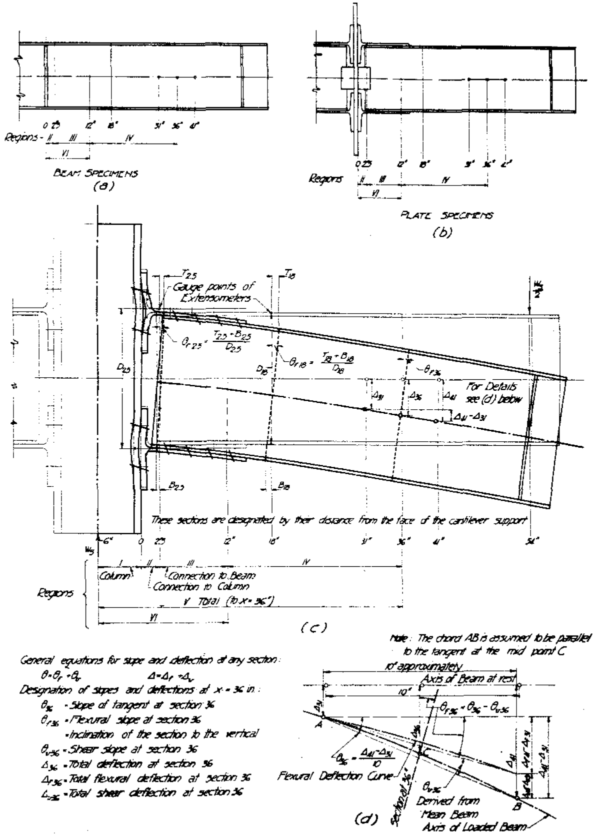


FIG. 14. *Regions, measurements and derived quantities, second series.*

14. *Flexural and Shear Slope at Any Section of the Mean Plain Beam*

Having determined the value of EI for the mean plain beam, the flexural slope at any section may be readily found from the first derivative of the deflection equation.

There being a constant shear between the load point and the ends of a specimen, and the value of EI being assumed as constant for the mean plain beam, the shear slope will be constant on either side of the load point. It was convenient to determine this slope by two methods, *(a)* and *(b)*.

Method *(a)* consisted in measuring the slope due to all causes at $x = 36$ in. and then subtracting from this the slope due to flexure. Deflectometer readings having been taken at $x = 31$, $x = 36$ and $x = 41$ in., the total slope at $x = 36$ is, as indicated in Fig. 14, the difference between the deflections at $x = 31$ and $x = 41$ in. divided by 10. The flexural slope at $x = 36$ in. may be calculated, and the difference, or shear slope, is applicable to any part of either half of the plain beam.

By Method *(b)* the deflection due to all causes was measured at $x = 36$ in. and from this the value of the flexural deflection, as found from the deflection equation, was subtracted. The difference when divided by 36 gave the shear slope throughout either half of the plain beam.

Values given by Method *(a)* were deemed to be more accurate than those given by Method *(b)*, since shortening of the stiffener under the central load between the point of support of the deflectometer bridge and the elastic line of the beam would cause the bridge to drop slightly with relation to the elastic line, thereby rendering the deflection readings too large. Dropping, or longitudinal tilting, of the bridge would not affect the accuracy of the results as obtained by Method *(a)*.

15. *Regional Division of a Specimen*

For purposes of analysis, and to show the influence of different portions of the connection on the deformation of the connection as a whole, it was found desirable to divide the specimen into four primary regions I, II, III and IV, illustrated for both cut and uncut specimens in Fig. 14. In referring to these it was sometimes found convenient to group all four into one region, V, and also I, II and III into VI. It should be noted that Region I exists only for column specimens, as it is the half width of the column. For plain beam and for plate specimens, therefore, Region VI comprises only Region II plus Region III. For uniformity, the nominal length of a connection, measured from its back or from the face of the support, along the beam was taken as 12 in., although the length of the riveted connection and of the bottom attachment of the other connections was slightly more than this.

16. *Flexural Slope Developed in Individual Regions of Fabricated Specimen*

Region I. While a cross section of the column stub is assumed to remain plane under loading, the deformation of the column flanges will cause a tilting of the connection, or a slope in the beam tangent at the face of the cantilever support. The flexural influence of Region I for any type of con-

nection is indicated by the difference in extensometer readings at $x = 2\frac{1}{2}$ in. for a column specimen and an equally loaded plate specimen utilizing that connection. In this difference there is included the difference between the behavior of the rivet group utilized for attaching the connection to a column flange and that of the corresponding rivet group used to attach the connection to a central plate. This difference may fairly be considered as an essential deformational characteristic of the half column width.

Region II. Flexural slope attributable to the deformation in Region II of the specimen will, if it be assumed that the 1-in. central transverse plate does not change thickness under load, be the slope developed in plate specimens in a distance of $2\frac{1}{2}$ in. from the face of the plate. It was impracticable, with the equipment available, to determine the slope developed in Region II of column specimens.

Region III. The flexural slope developed in Region III is the difference between the flexural slope developed in $x = 12$ in. and that developed in $x = 2.5$ in.

Since the gauge points to the longer extensometers were placed at $x = 18$ in., so as to be clear of local disturbance near the end of the connection, it was necessary to find the flexural slope developed in $x = 12$ in. by subtracting from that developed in $x = 18$ in. the flexural slope developed in the 6 in. between the two cross sections involved. This difference of slope may be taken as the same as is developed between the corresponding sections of the mean plain beam under equal load.

The adjustment was made by writing expressions for the flexural slope at $x = 12$, $x = 18$ and $x = 36$ in. in the plain beam and then relating the first to the other two.

Region IV. The flexural slope developed in Region IV is the difference between that developed in $x = 36$ in. and that developed in $x = 12$ in.

Region V. The total flexural slope developed in Region V, that is in 36 in. from the back of the connection, was found by summing the effects for the component regions.

Region VI. Region VI comprises the entire connection, and extends from $x = 0$ to $x = 12$ in. The flexural slope developed in it is, therefore, the sum of those developed in Regions I, II and III.

17. *Increment in Flexural Deflection at Any Point Due to Change of Slope in Any Region Between the Point and the Cantilever Support*

It was necessary to determine the deflections produced at $x = 36$ in. as a result of the flexural deformation of any selected region.

The contribution to flexural deflection at N made by the flexural deformation of any region, R, of a specimen may be expressed as the product of the flexural slope angle developed in the region and the distance from N to the intersection P of the tangents drawn to the flexural elastic curve at the two ends of the region. This is evident from Fig. 15.

If, now, it is assumed for the time being that the moment of inertia in Region R is constant, the flexural deflection at u_2 may be indicated by the ordinary deflection formula. The first derivative of this gives the slope, which was determined experimentally. An expression for the average moment of inertia of Region R in terms of known quantities is then obtainable. Utilizing this in the deflection equation, the deflection at N due to flexure in Region R is expressible as the product of the slope angle developed in the region and a determinable distance K_R.

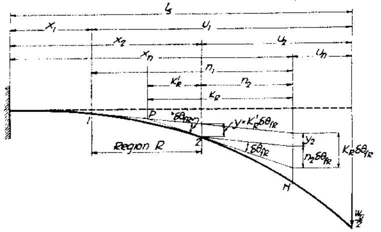


FIG. 15. *Influence of flexural deformation of a region on flexural deflection at any point.*

18. *Derivation from Cantilever Specimens of Flexural Deflection at Mid-span of 19-ft. Simulated Beam*

In computations of story drift due to wind, a knowledge of the probable flexural deflection of the point of inflection of a beam subjected to wind moment from a normal to the unbent column at the connection is required. If the clear span of the beam be 19 ft. and the point of inflection be assumed to be at mid-span, then the flexural deflection at $x = 114$ in. is the desired quantity.

In estimating the behavior of a 114-in. cantilever loaded at its end on the basis of the performance of a 54-in. cantilever similarly loaded, account must be taken of the fact that for the same moment at the back of the connection, the moment at the centre of any region of the connection would, for a wind shear acting at 114 in., be different from that existing in a test specimen with the reaction acting at 54 in. from the back of the connection. When the moment at the latter point is the same as that for any selected load stage on the test specimen, the moment at the middle of Regions I, II and III will be respectively 0.98, 1.01 and 1.08 times as great as it would be for these regions in the test specimen, while the shear would be 54/114 or 0.4735 as great.

Utilizing the regional slope angles, $\delta\theta_f$, found for the specimen, and, noting that the deflection at $x = 114$ in. due to the bending of the region between $x = 12$ and $x = 114$ in. is merely the deflection of a plain beam cantilever from a tangent at $x = 12$, the flexural deflection at $x = 114$ in. may be computed.

19. *Shear Deflection at Any Section of the Mean Plain Beam*

The flexural deflection at any section of the mean plain beam being readily calculable, once the correct value of EI has been determined, the shear deflec-

tion is simply the difference between the measured total deflection and the computed flexural deflection.

20. *Increment of Shear Deflection in Any Region of Mean Plain Beam*

For a constant shear and uniform section the shear slope angle θ_v, is constant and therefore the shear deflection at any distance from the midpoint of the specimen is this distance multiplied by θ_v.

21. *Increment of Shear Deflection in Individual Regions of Fabricated Specimen*

Region I. Since Region I exists only for column specimens, the shear deflection in this region will merely be the difference between the shear deflection at the same point for the plate specimen, similarly loaded.

Region (II + III). As the available equipment did not permit the differentiation of shear deflection in Regions II and III, the plotted results (see Section IV) show these regions combined for shear. Plate and column specimens being alike for Region (II + III), and the shear deflection in Region IV being assumed the same as for the same region of the similarly loaded mean plain beam, then for either plate or column specimens, the shear deflection for the combined region is the shear deflection for the *plate* specimen at x = 36 in., less the shear deflection between x = 12 and x = 36 in. for the mean plain beam.

Region IV. Beyond the connection the shear deflection for Region IV is that for an equal length of mean plain beam.

Region V. The increment of shear deflection arising in Region V of a fabricated specimen is obviously the difference between the total deflection in x = 36 in. and the flexural deflection in the same distance.

Region VI. For Region VI the increment of shear deflection may be conveniently expressed as the increment for Region V, less the shear deflection in Region IV.

22. *Derivation from Cantilever Specimens of Shear Deflection at Mid-span of 19-ft. Simulated Beam*

Utilizing the regional shear deflections found for the test specimens, and noting that the shear deflection for the region from x = 12 to x = 114 in. is 102/24 that for Region IV under the same shear, the shear deflection in 114 in. of the simulated beam is readily found. Allowance must be made, of course, for the fact that the shear is applied at x = 114 in. for the simulated beam and at x = 54 in. for the specimen.

23. *Coefficient of Restraint*

One of the objects of the investigation was the determination of the relation existing between the moment M_e developed at the supports of a uniformly loaded beam with constant moment of inertia, and provided with various types of connections, and the moment M_r at the supports of the same beam if it were perfectly restrained. Let this relation be called the coefficient of

restraint and be designated as R. Then, if W be the total load on the span and l the span,

$$M_s = RM_r = -R \cdot \frac{Wl}{12} = -R \cdot \frac{Wl^2}{12} \quad (5)$$

If $R = 1$, the restraint is perfect; if it be zero the beam is simply supported only, and hence $M_s = 0$. The value of R is consequently a direct index of the merit of a connection, so far as its ability to introduce negative moment at the end of a beam and to lessen the positive moment at mid-span, or elsewhere, is concerned.

In lessening the moment at the centre of a beam, the value of restraint is evidenced by the extent to which the points of inflection are shifted towards mid-span and the "suspended span" thereby shortened. The effect of resistance to end tilting on the position of the point of inflection is augmented by welding or riveting connection material to the beam near the ends. An effective coefficient of restraint greater than unity may thus result.

The correct effective coefficient of restraint is obtained by considering the difference between the slope angles developed in the plain beam and the cut-and-connected specimen out to a certain point clear of the connection as arising wholly at the contact of the connection with the support. It is immaterial as to how or where this difference was created.

The coefficient of restraint of a beam more or less restrained at the ends may be shown to depend on the ratio of the slope, θ_{fo}, of the elastic line at the support of a beam of similar but constant cross section to the theoretical slope, θ_s, at the support of the same beam simply supported and similarly loaded.

Since θ_{fo} is the slope at the support of a fictitious beam it is not directly measurable, but it is equivalent to the difference in flexural slope, at any section beyond the connection, between the simulated uncut and the cut-and-connected beams under equal loads. It may be positive or negative and is obtained as follows:

For specimens of the first series this angle was obtained for each specimen from the original observations according to Equations (2), (3) and (4) in Art. 12.

For each type of specimen of the second series the mean angle was obtained by using the tabulated values for the mean curves, described in Art. 7, in the following equations:

Determination by the 18-in. extensometers,

$$\text{Plate specimens,} \quad \theta_{fo} = \theta_{f18\,(P-B)} \quad (6)$$

$$\text{Column specimens,} \quad \theta_{fo} = \theta_{f18\,(C-B)} \quad (7)$$

Determination by 31-in. and 41-in. deflectometers,

$$\text{Plate specimens,} \quad \theta_{fo} = \theta_{36\,(P-B)} \quad (8)$$

$$\text{Column specimens,} \quad \theta_{fo} = \theta_{36\,(C-B)} \quad (9)$$

Since the shear slope included in θ_{36} results from the shear deflection between $x = 31$ and $x = 41$ in., and since the section in this region is alike in all specimens, this shear slope has been assumed to be the same for all specimens (see Fig. 14).

In the following paragraph pertaining to restraint, which does not involve shear slope, the subscript fo has been dropped and θ is understood to represent θ_{fo} as described in the preceding paragraphs.

The relations of these angles to the coefficients of restraint and to support and centre moments are shown in Fig. 16.

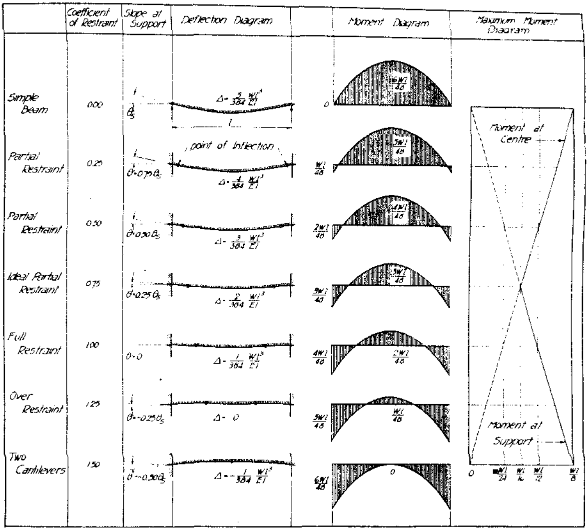


FIG. 16. *The relation of coefficient of restraint to slope angle, deflection and moment distribution.*

Coefficients of restraint in excess of 1.0 are possible if the conditions in the adjacent spans cause the elastic line at the supports of the span considered to slope upwards, or if the stiffening effect of the connections within the span similarly develop a negative value of θ as defined above.

For a partially or wholly restrained beam, uniformly loaded, and of constant moment of inertia, the moment at the support, applying the basic slope-deflection is,

$$M_s = \frac{2EI\theta}{l} - \frac{wl^2}{12} \tag{10}$$

where E is the modulus of elasticity of the material, I the moment of inertia of the beam, l the span and w the uniform load per unit of length.

Now the slope angle θ may be replaced by $K\theta_s$, where K is a coefficient varying with the end moment. Hence

$$\theta = K\theta_s \tag{11}$$

From Equation (10) the value of θ_s may be found by equating the support moment to zero. It is

$$\theta_s = \frac{wl^3}{24EI} \tag{12}$$

Consequently

$$\theta = \frac{Kwl^3}{24EI} \tag{13}$$

Introducing in Equation (10), the values of M_s and M_r, and simplifying we have

$$R = (1 - K) = 1 - \frac{\theta}{\theta_s} \tag{14}$$

Thus, it is evident that the coefficient of restraint R and the ratio θ/θ_s, or K, are complementary, that is either one is equal to unity minus the other.

In a partially restrained beam the moment at the support, as is evident from Equation (5), varies directly as the coefficient of restraint when the load is constant, and as the load, when the coefficient of restraint is constant. Therefore, if it is desired to maintain the same moment with a varying coefficient of restraint, the required load will increase in the same ratio as the coefficient of restraint decreases, and *vice versa*. In other words, the load will need to vary as $\frac{1}{R}$.

The above relations have reference to symmetrical, uniformly loaded beams with constant moment of inertia and with the same degree of restraint at the two ends. It was not considered practicable to test full-size beams of this type, and so the experimental work was carried out with specimens of the double-cantilever type. The relation between load and moment, and therefore angular deformation, found for the cantilever specimens must now be adjusted or transformed in such a way as to be applicable to the simulated partially restrained beams, that is, to beams that were being indirectly investigated by study of the behavior of equivalent, more readily manageable, specimens.

Consider now that a double-cantilever specimen with two arms of length l_s each, Fig. 12, is subjected to a load of W_s. For the moment $\frac{1}{2}\ W_s\ l_s$ at each cantilever support there will be a certain slope angle θ corresponding to a coefficient of restraint R, as yet unknown. Obviously, if the tested connection existed at either end of the beam represented or simulated by the cantilever specimen, and the moment and shear conditions were the same within the limits of the connection, the slope angle and the coefficient of restraint would be the same as were found for the cantilever specimen. The problem, then, is to determine the load that would need to be applied to the partially restrained simulated beam in order that the moment at the face of the support, the slope angle θ, and the coefficient of restraint R, will be the same as for the test specimen.

Now the moment at the cantilever supports of the test specimen is $\frac{1}{2} W_s l_s$, and is independent of the slope angle θ, so long as the specimen remains intact. If the load required on the partially restrained beam be W and the span length be l, then, for equal moments,

$$R \frac{Wl}{12} = \frac{W_s l_s}{2}$$

from which

$$W = \frac{6 W_s l_s}{Rl} \quad . \tag{15}$$

This load W applied to the partially restrained beam produces a slope angle θ at the support and if applied to a simply supported beam would produce an angle θ_s at the support. Hence the coefficient of restraint is, as indicated in Equation (14), $R = 1 - \theta/\theta_s$.

Now the slope angle at the support of a simply supported beam varies directly as the load, within the elastic limit, and if the load became W_s, the same as on the double cantilever specimen, the support angle (Equation (12)) would be some proportionate angle, with a value

$$\theta'_s = \frac{W_s l^2}{24EI} \cdot \tag{16}$$

By proportion, then, we have the relation

$$\theta_s = \frac{W}{W_s} \theta'_s \tag{17}$$

Introducing the value of W from Equation (15),

$$\theta_s = \frac{6l_s}{Rl} \cdot \theta'_s \tag{18}$$

and, from Equation (14),

$$R = 1 - \frac{Rl}{6l_s} \cdot \frac{\theta}{\theta_s'} \quad .$$

Solving for R, the coefficient of restraint becomes

$$R = \frac{6 \, l_s \, \theta'_s}{6 l_s \theta_s' + l\theta} \quad . \tag{19}$$

If, as was adopted in the first series, the span, l, of the simulated beam is the ideal value $l' = 6l_s$, and the slope, θ'_s, at the support of the beam simply supported under load W_s is θ_s'', R becomes

$$R' = \frac{\theta_s''}{\theta_s'' + \theta} \quad . \tag{20}$$

For any other span of simulated beam, l, with the corresponding slope, θ'_s, Equation (19) may be written

$$R = \frac{\frac{l'}{l} \theta'_s}{\frac{l'}{l}\theta'_s + \theta} \quad , \tag{21}$$

which, for specimens of the second series where

$$\frac{l'}{l} = \frac{6 \times 54}{228} = 1.42,$$

becomes

$$R = \frac{1.42\,\theta'_s}{1.42\,\theta'_s + \theta} \,. \tag{22}$$

From Equation (16) it will be seen that the support angle varies as the square of the span. Making use of this fact Equation (21) may be readily transformed to show the effect of the span length of the simulated beam on the coefficient of restraint.

The load required to produce the same end moment and the same end slope angle, θ, in the partially restrained beam as is produced in the specimen by a load W'_s is then, by Equation (15),

$$W = \frac{6l_s}{l} \cdot \frac{W'_s}{R} \,, \tag{23}$$

where R is determined by the applicable equation, (20), (21) or (22).

24. *Story Drift Due to Wind*

One of the principal objects of the investigation was to determine the relative drift or horizontal movement under wind, or other lateral force, of a given story of a building with various types of connections between the beam and the columns.

Since for a symmetrical bent, symmetrically loaded, gravity loads produce *no lateral movement, the only influence that gravity load would have on* drift for bents of the type mentioned would be in the possible difference in connection deformations, when subjected to a given load increment at different stages in their safe loading range. Neglect of this circumstance is not likely to lead to any appreciable error when welded connections are used. The error is greater for riveted connections, as may be seen from an examination of Fig. 10 *(b)*, *(f)* and *(j)*.

In the present investigation, detailed consideration has been given to two cases of loading:

(1) Wind acting in one direction up to the safe capacity of the connections;

(2) Wind to capacity in one direction, reversing to capacity in the opposite direction.

In comparing the story drifts to be expected when different types of connections are used it must be borne in mind that the total story drift angle α is the sum of three drift angles α_1, α_2 and α_3 as follows:

α_1, caused by flexural and shear deflection of the beams unaffected by connections;

α_2, caused by the effect of the connections on beam deflection;

α_3, caused by the flexural and shear deflection of the columns.

Evaluation of the effect of the connections is, therefore, significant only when compared with the total effect of the three causes. Obviously, the bending in the beams and columns depends on the sections chosen, and the results are consequently only those for a particular, though typical, case.

Connections of Asymmetric Deformation. A connection is considered to be symmetrical if its top and bottom attachments are identical and the processes of connecting them are similarly carried out. While the individual attachments may deform quite differently in tension and compression, the deformation of the connection as a unit will be symmetrical, that is, equal normal and reversed loads will produce equal deflections in opposite directions.

If, either in design or fabrication, a connection is not symmetrical it may not deform symmetrically under loads in opposite directions. If the stronger or stiffer attachment is proportionately stronger than the other both in tension and compression symmetrical deformation will result. Therefore only when one attachment is disproportionately stronger, or weaker, than the other in one direction, will the connection as a unit deform asymmetrically.

The greatest difference in deformation for opposite loads among the connections tested occurred for the one type of symmetrical design—the riveted connection. The top flange was disproportionately weaker in tension. It is thought that this was a result of the order of riveting, and excessive stresses in the inner tension rivets due to T-flange deformation at maximum loads.

Displacement of the Point of Inflection. Since the connections at opposite ends of a beam, under wind load only, are deformed in opposite directions, any lack of symmetry in the deformation occurring will displace the point of inflection in the beam from the centre towards the critical connection, or that one which is loaded in its weaker direction. This eccentricity will be greatest for a single bent and will be reduced in any bent of a series by the effect of the adjacent bents.

The displacement of the point of inflection corresponding to the lack of symmetry in the extreme case cited above was less than 2% of the half-span and was therefore considered negligible. If there had been, however, a marked difference in the behavior of the connection under loads in opposite directions, it would have been necessary to modify the loading program adopted, use the suitable mean curves provided thereby, and allow for the displacement in the point of inflection in the calculation of story drift angle. In view of the existing circumstances, however, the much simpler method of calculating story drift angles based on the assumption of a central point of inflection was adopted.

The story drift angle, α_{1+2}, due to the deformation of the beam and its connections for any given moment at the support caused by a wind load is equal to the deflection, $\triangle$, of the simulated beam at mid-span divided by half the span length, or

$$\alpha_{1+2} = \frac{2\triangle}{l}\,. \tag{24}$$

The value of $\triangle$ used for any given connection is that calculated for the corresponding test specimen with the same end moment as for the simulated beam and with an assumed cantilever arm of 114 in., or half the span of the simulated beam. That is,

$$\alpha_{1+2} = \frac{2\triangle_{114}}{228} = \frac{\triangle_{114}}{114}\,. \tag{25}$$

The drift angle, α_2, attributable to the connections, may be determined by using the difference in deflection between uncut, and cut-and-connected specimens, that is, for plate connections,

$$\alpha_2 = \frac{\Delta_{114\,(P\,-\,B)}}{114} \tag{26}$$

and for column connections,

$$\alpha_2 = \frac{\Delta_{114\,(C\,-\,B)}}{114} \quad . \tag{27}$$

The drift angle, α_3, due to column bending is determined by the deflection of the column at mid-height. It is

$$\alpha_3 = \frac{2\,\Delta_h}{h} \quad , \tag{28}$$

where Δ_h is the deflection of the column at mid-height and h is the height of the story = 144 in.

Summing the effects, the total drift angle becomes

$$\alpha = \alpha_1 + \alpha_2 + \alpha_3 \tag{29}$$

which, for cases where the connections are to the column web in the simulated story, becomes

$$\alpha = \frac{\Delta_{114P}}{114} + \frac{\Delta_h}{72} \tag{30}$$

and where the connections are to the column flanges becomes

$$\alpha = \frac{\Delta_{114P}}{114} + \frac{\Delta_h}{72} \quad . \tag{31}$$

For reasons given in Art. 7 the XY and Z loads were chosen to represent the behavior of the connection when subjected to repeated reversals of load and repeated loads in one direction, respectively. Consequently, in computing the drift angles resulting from these two types of wind loads the values of Δ_{114} were derived from the tabulated results for the mean XY and Z curves respectively.

(To be concluded in next issue)

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THE RELATIVE RIGIDITY OF WELDED AND RIVETED CONNECTIONS[1]

BY C. R. YOUNG AND K. B. JACKSON

IV. Analysis of Experimental Results

25. *General*

The specimens of the first series were designed to develop beam continuity only, and tested to determine the relation between load and coefficient of restraint in the simulated beam. The results obtained are given in Figs. 26 to 30 and discussed, together with similar results for the second series, in Arts. 28 and 29, on Coefficient of Restraint.

The specimens of the second series were designed to develop beam continuity and to resist wind load moments. They were tested, not only to determine their over-all capacity in these respects, but also to allocate the particular causes of strength or weakness.

The results for the second series therefore include:

(a) The deformation of the test specimens;

(b) The coefficient of restraint and moment distribution in the simulated beam;

(c) The story-drift angle in the building of which the simulated beam would constitute a part.

Finally, a table of ultimate strengths and factors of safety, etc., is given for the specimens of each series.

26. *Deformation of the Test Specimens, Second Series—Flexural Slope by Flanges and Regions*

For purposes of analysis the connections have been divided longitudinally into three regions, I, II and III, and transversely into two halves termed the top and bottom flanges, as indicated in Fig. 14. Regions IV and V extend beyond the connection and Region VI comprises I, II and III.

The flexural slopes developed in each flange and region have been computed for the two significant loads, XY and Z, as described in Sections II and III, and are presented in Figs. 17 to 23. Figs. 17 and 18 give, in statistical form

[1] *Concluded from July issue.*

for each and both flanges and for each and all regions wholly comprised within the limits of the connection, the total slopes developed by the XY and Z loads, respectively. Figs. 19 and 20 indicate by curves the development of flexural slope during the application of the two loads, for each flange and

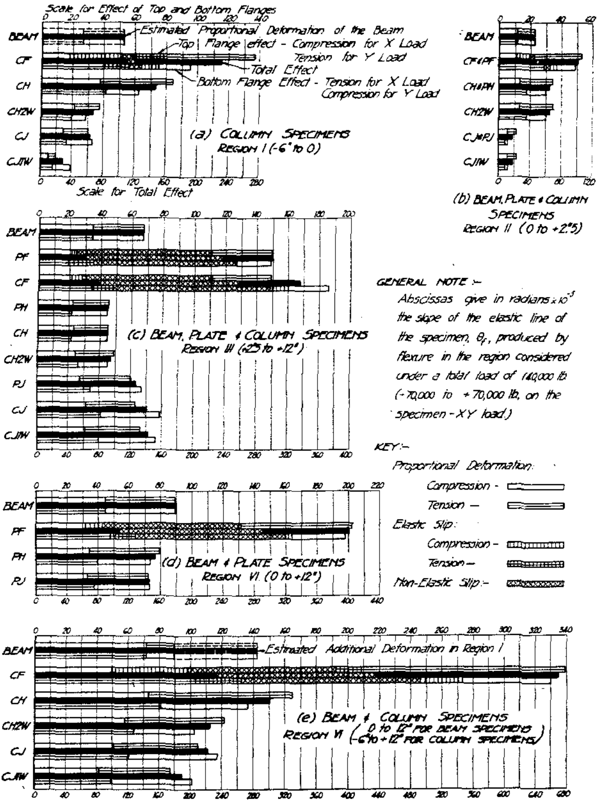


FIG. 17. *Flexural slope produced in Regions I, II, III and VI of specimens by total load change of 140,000 lb. (−70,000 to +70,000 lb.).*

region, and Figs. 21, 22, and 23 give the sum of these regional developments for each flange and for both flanges.

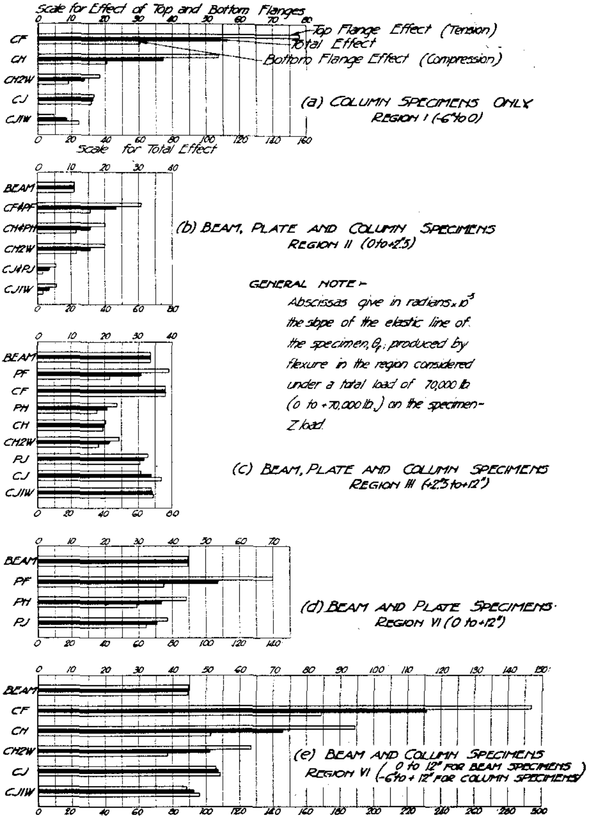


FIG. 18. *Flexural slope produced in Regions I, II, III and VI of specimens by total load change of 0 to +70,000 lb.*

The flexural slope developed in a given flange and region is determined by:
(1) The type of connection—*PF*, *CH2W*, etc.;

(2) The flange included—top or bottom;

(3) The stress involved—tension or compression;

(4) The loading sequence—including or excluding non-elastic slip.

In some types of connections the effect of a change in (2), (3) or (4) is small, while in others it is considerable. A comprehensive test, therefore, of any type of connection must involve both flanges in both kinds of stress and the loading sequence must be such as to include any non-elastic slip that may

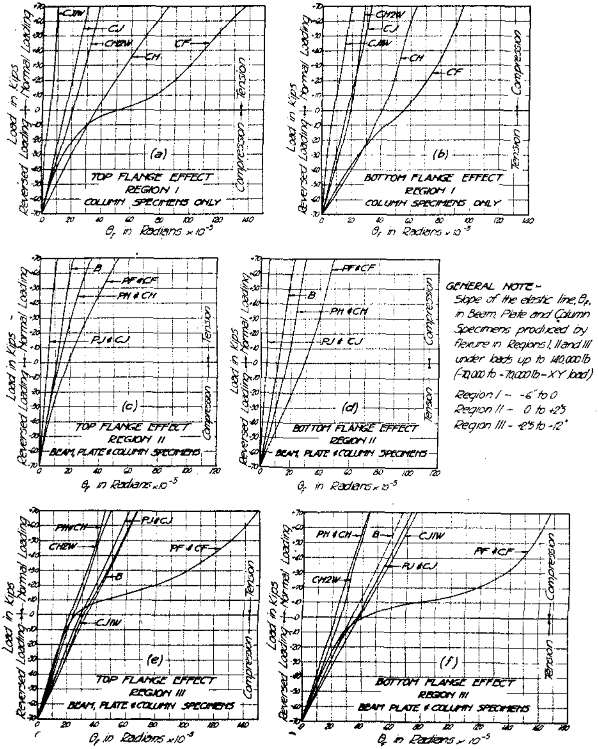


FIG. 19. *Load-slope relation for specimens produced by flexure in Regions I, II and III under load varying from −70,000 to +70,000 lb.*

occur. This is provided by the XY load, and the corresponding flexura slopes developed under it in all regions by all types are indicated by th solid black lines in Fig. 17. The relative values of these slopes are given i Table II, which provides the simplest means of comparing the regional an over-all flexural performance of the connections and the plain beam. Th

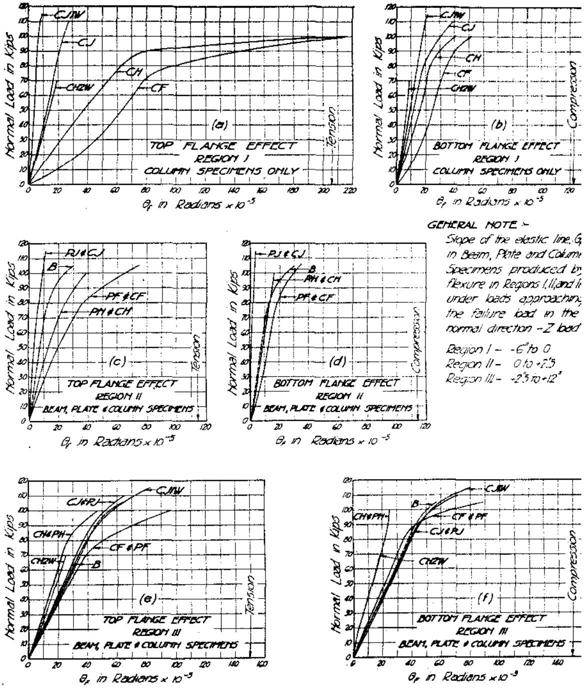


FIG. 20. *Load-slope relation for specimens produced by flexure in Regions I, II and III under load varying from 0 to + approximate failure load.*

relative slopes developed in the connections are given in three forms in th table:

(a) In terms of the slope developed in the plain beam in the same region—to compare the types in each region;

TABLE II

RELATIVE FLEXURAL SLOPES DEVELOPED BY TWO FLANGES IN EACH REGION FOR EACH TYPE OF CONNECTION OF THE SECOND SERIES DURING A COMPLETE REVERSAL OF LOAD*

Connection				Relative flexural slope											
Type	Attached to column web or flange	With or without column stiffeners	Specimen	*(a)* Beam slope in each region =1.00				*(b)* Total beam slope = 1.00				*(c)* Total slope in each specimen =1.00			
				Region No.				Region No.				Region No.			
				I	*II*	*III*	Total VI	I	II	III	Total VI	I	II	III	Total VI
Plain beam	—	—	*B*	—	1.00	1.00	1.00	—	0.25	0.75	1.00	—	0.25	0.75	1.00
	Assumed as extended into Region No. 1			1.00	1.00	1.00	1.00	0.59	0.25	0.75	1.59	0.37	0.16	0.47	1.00
Riveted T	Web	—	*PF*	—	2.27	2.22	2.24	—	0.57	1.67	2.24	—	0.25	0.75	1.00
	Flange	Without	*CF*	2.19	2.27	2.50	2.34	1.30	0.57	1.87	3.74	0.35	0.15	0.50	1.00
Welded T	Web	—	*PH*	—	1.42	0.67	0.86	—	0.36	0.50	0.86	—	0.42	0.58	1.00
	Flange	Without	*CH*	1.38	1.42	0.67	1.05	0.83	0.36	0.50	1.69	0.49	0.21	0.30	1.00
		With	*CH2W*	0.62	1.42	0.70	0.79	0.38	0.36	0.52	1.26	0.30	0.29	0.41	1.00
Welded gusset	Web	—	*PJ*	—	0.38	0.95	0.81	—	0.10	0.71	0.81	—	0.12	0.88	1.00
	Flange	Without	*CJ*	0.60	0.38	1.05	0.78	0.36	0.10	0.79	1.25	0.29	0.08	0.63	1.00
		With	*CJ1W*	0.28	0.38	1.06	0.66	0.17	0.10	0.80	1.07	0.16	0.09	0.75	1.00

* See "*Total effects*", *Fig. 17.*

(b) In terms of the *total* slope developed in the plain beam—to compare 11 types in all regions on a common basis;

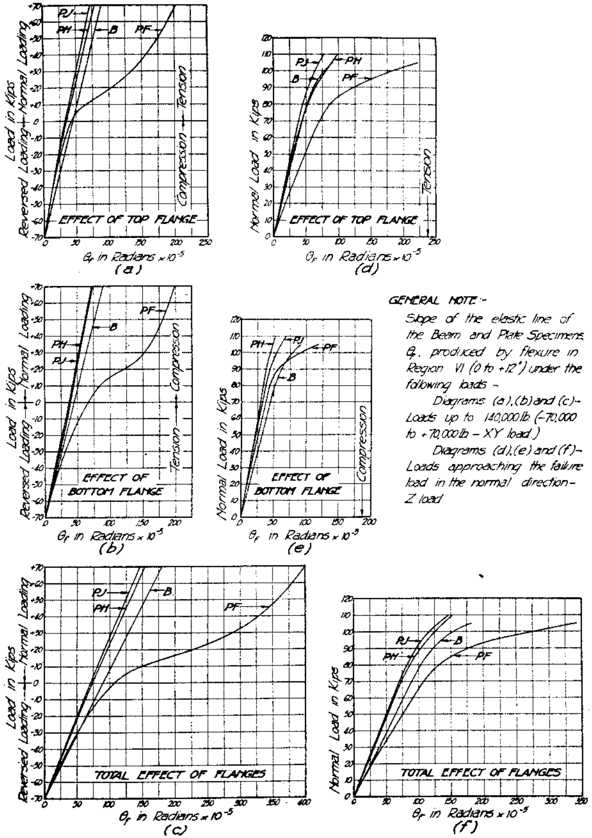
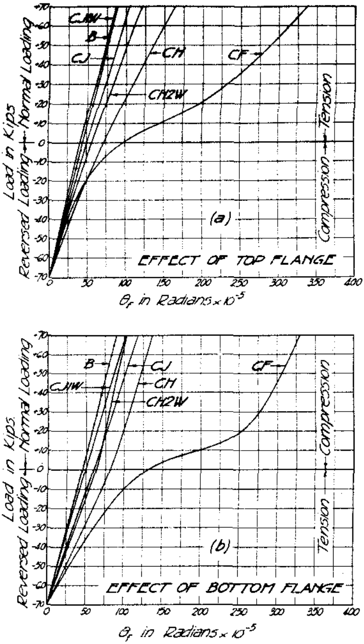


FIG. 21. *Load-slope relation for beam and plate specimens produced by flexure in Region VI under loads varying from −70,000 to +70,000 lb. and from 0 to + approximate failure load.*



GENERAL NOTE:—
Slope of the elastic line of the Beam and Column Specimens, θ_r, produced by flexure in Region VI (0 to +12" for Beam Specimens B and -6" to +12" for Column Specimens CF, CH, CH2W, CJ and CJIW) under loads up to 140,000 lb. (-70,000 to +70,000 lb. —XY load)

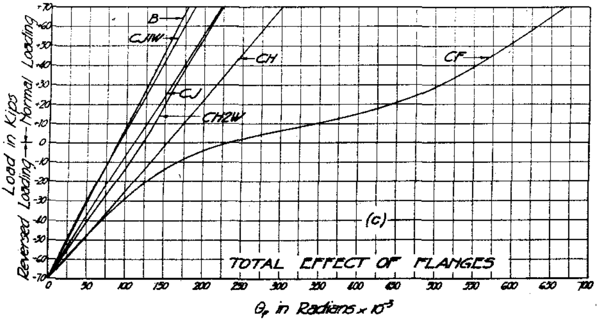


FIG. 22. *Load-slope relation for beam and column specimens produced by flexure in Region VI under load varying from −70,000 to +70,000 lb.*

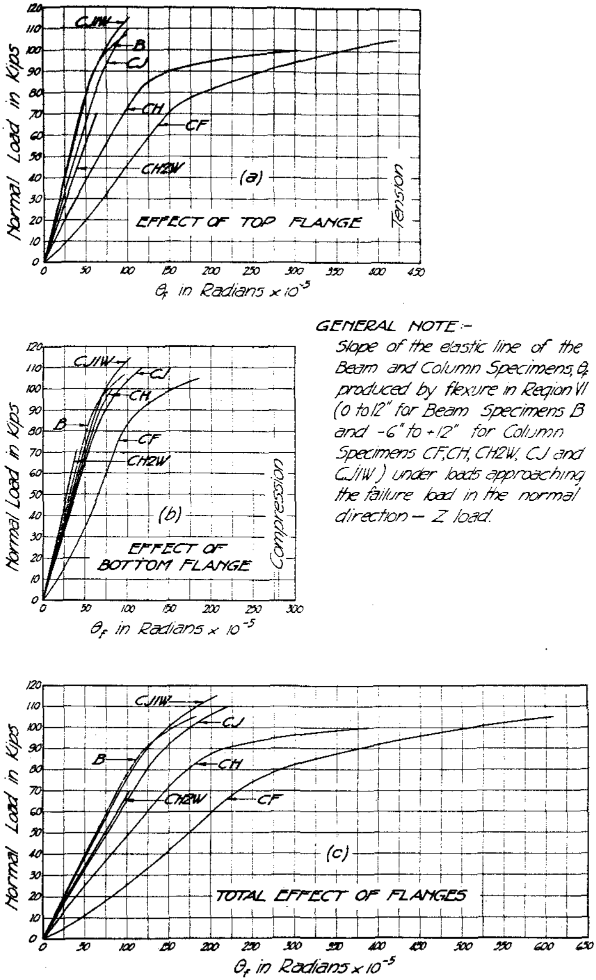


FIG. 23. *Load-slope relation for beam and column specimens produced by flexure in Region VI under loads varying from 0 to + approximate failure load.*

(c) In terms of the total slope in each type—to compare the regions in each type.

To make the comparison complete it is necessary to estimate the slope that would be developed in the plain beam if it were to extend through Region I. The relative values of this slope as required in *(a)*, *(b)* and *(c)* are indicated in the second line of the table.

The slope developed in the top and bottom flanges respectively, is shown above and below the solid black lines in Fig. 17, and these flange deformations are further analyzed, by reference to the load-slope curves in Figs. 19 to 23, to determine the relative significance of tension, compression and elastic and non-elastic slip. That portion occurring during the *X* load may consist of proportional deformation only, indicated by a straight load-slope curve, or it may include elastic slip indicated by a more or less distinct "toe" in the curve. This characteristic is most prominent in *F* specimens in Regions I and III, as is evident from Fig. 19.

The deformation during the *Y* load includes non-elastic slip, if any occurs, the value of which is estimated by the difference between the deformation occurring during the *Y* and *Z* loads (within the same load range), allowance being made for any excessive "subsequent set" during the *Z* load. Elastic slip during the *Y* load is assumed to be the same as in the *Z* load, which is indicated by a toe in the *Z* load curve. The method of estimating these components of the deformation in a single flange is illustrated in Fig. 11 *(b)* and the results are indicated by a subdivision of the flange deformations shown in Fig. 17.

This analysis of the flexural deformation occurring in each flange and region of the specimens under design load (Fig. 17), combined with the evidence obtained during failure loads, forms the basis of the following observations which attempt to explain the deformations indicated in terms of the structural details involved.

Region I. Region I consists nominally of half the column width but, as the deformation indicated is derived from the differences in short-extensometer readings on plate and column specimens, it includes any difference in the deformation in Region II caused by the column, and, as pointed out in Art. 16, any difference in the behavior of the rivet group utilized for attaching the connections to a column or connections to one another through a plate.

(1) General

(a) The two connections utilizing T's and no column flange stiffeners, *CF* and *CH*, produce deformations in the column 3.6 and 2.3 times as great respectively as that produced by the gusset plate connection, *CJ*. Fig. 17 *(a)*.

(b) The tension attachment to the column is the critical element in the *CF* and *CH* connections. See Fig. 20 *(a)*.

(c) The column flange stiffeners used in *CH2W* and *CJ1W* reduced the deformation in the column to 45% of its previous value, being more effective in tension than in compression.

(2) The Riveted T, or *CF*, Specimens

(a) Elastic slip is slightly greater in compression than in tension, but is about twice as great in the top flange as in the bottom. Elastic slip here is assumed to be caused by progressive seating, that is by the bending of the column flanges, inward in compression until the centres of the column and T flanges are in contact, and outwards in tension until the edges of the T flanges bear against the column flanges and greatly restrain further bending in both column and T flanges. If this is true the amount of elastic slip would depend upon the distances between the surfaces at zero load, which might well be affected by the sequence in riveting. Otherwise, the greater elastic slip in the top flange can be explained only by accidental variations in the curvature of the bearing surfaces after riveting.

(b) Proportional deformation is 2.6 times as great in tension as in compression but only slightly greater in the top flange than in the bottom. This excessive deformation in tension is due to the outward bending of the column flanges and the elongation of the connecting rivets.

(c) By comparing the deformations at 100,000 lb. during the *Z* load, Fig. 20, it will be seen that the tension-half of this region is the critical element in this type of connection.

(3) The Welded T, or *CH*, Specimens

(a) A negligible amount of elastic slip occurs in compression during the seating of the T and column flanges.

(b) The greater concentration of the connecting material about the column flange in this type, compared with the previous one, produces less proportional deformation in tension on account of the decrease in column flange bending, but greater deformation in compression.

(c) The outward bending of the column flanges in line with the tension connection causes excessive concentration of stress in the centre of the horizontal welds between T and column flanges. Progressive failure began here within the design load and as a result complete failure occurred at an early stage. The column flange stiffeners, obviously, improved this defect by reducing the bending of the column flanges, but similar though less serious concentration of stress occurs in the vertical welds due to the bending of the T flanges, and it is this inevitable, uneven distribution of stress in the welds connecting the T to the column that is responsible for the low failure loads obtained for this type of connection.

(4) The Welded Gusset Plate, or *CJ*, Specimens

(a) Slip does not occur.

(b) Proportional deformation is practically the same in tension and compression but slightly greater in the bottom flange than in the top, owing to the altered distribution of stress caused by the shelf plate required in the bottom connection. The column flange stiffeners reduced top flange deformation to one-third its previous value and bottom flange to nearly one-half.

Region II. The deformations in this region are assumed to be the same in plate and column specimens and are derived from the short-extensometer readings on the plate specimens: they include; longitudinal deformation of the attachment within $2\frac{1}{2}$ in. of the column face, bending of the T flanges if any, and deformation of the connecting welds or rivets.

(a) The mean cross-sectional areas of the two attachments in this region do not vary by as much as 8% in the three types of connections, and consequently the wide variations in deformation ($F : H : J = 6 : 4 : 1$) must be attributed to the bending of the T flanges in *CF* and *CH* and the deformation of the rivets in *CF*. This assumption is supported by the facts that the deformation in tension is much greater than in compression, (60 : 40), and that elastic slip occurs in compression in these two types.

(b) The deformations of top and bottom flanges are very similar in the *F* specimen, for which the top and bottom attachments are the same, but the greater differences in the *H* and *J* specimens are to be expected, on account of the bottom attachments being heavier than the top ones. Another reason for the small deformations indicated in the bottom flange, involving stress distribution and the position of the gauge points, will be discussed later.

Region III. The deformations indicated for this region are derived from the differences in readings of the short and long extensometers, and include the longitudinal deformation of the attachment and beam, and the shear deformation of the connecting rivets or welds with any slip that may occur.

(a) As might be expected, the deformations in this region are similar in plate and column specimens of the welded types, and are not affected by the addition of column flange stiffeners. In the riveted specimens, where slip and elastic deformation are greatly affected by details in the process of riveting, slight variations of no significance occur.

(b) Non-elastic slip produces more than half the deformation that occurs in the riveted specimens in this region and constitutes nearly one-third of the total deformation of this type of connection.

(c) Elastic slip is negligible.

(d) Proportional deformation is similar to that of the plain beam, except in the welded T specimens where it is reduced by the stiffening effect of webs of the T's on the beam flanges.

Region VI. The regional analysis given above is based on the assumption that the cross-sectional planes through the gauge points of the extensometers remain plane during loading and therefore the movement of the gauge points accurately represents the inclination of the planes. Owing to the uneven distribution of stress in the vicinity of the gauge points of the short extensometers the assumption here is only approximately true, and there are obvious reasons for believing that some of the deformation charged to one region actually occurs in another. It is thought, however, that the gauge points of the long extensometers are removed sufficiently far from the causes of uneven stress distribution to be unaffected by them, and consequently the deform-

ation indicated for Region VI, based solely upon the long-extensometer readings and the adopted value of the deformation of the plain beam between the end of the region and the extensometer gauge points, is thoroughly reliable. The deformations indicated in Regions I, II and III are less accurate subdivisions of this deformation.

The analysis of the deformation is indicated in Fig. 17 *(d)* and *(e)* and agrees with the corresponding curves in Figs. 21, 22 and 23 and the regional analysis given above.

Table III indicates the total flexural deformation that occurs in each type during the *XY* load and the relative effect of tension and compression, top and bottom flanges, and normal and reversed loads in producing it.

From these results it is apparent that:

(1) About 30–45% of the deformation of the riveted types consists of non-elastic slip.

(2) The aggregate elastic deformation in tension is from 4–33% greater than that in compression for welded specimens, and from 56–70% greater for the riveted specimens.

TABLE III

ANALYSIS OF FLEXURAL DEFORMATION THAT OCCURS IN REGION No. VI DURING THE *XY* LOAD

Type of connection	Total flexural deformation in radians $\times 10^{-5}$	Percentages of total deformation							
		(a) Stress			*(b)* Flange		*(c)* Load		
		Tension	Compression	Non-elastic slip	Top	Bottom	Normal	Reversed	Non-elastic slip
PF	400	34	20	46	50	50	27	27	46
PH	154	55	45	—	52	48	51	49	—
PJ	146	53	47	—	50	50	51	49	—
CF	669	42	27	31	51	49	34.5	34.5	31
CH	301	57	43	—	55	45	49	51	—
CH2W	224	57	43	—	54	46	46	54	—
CJ	222	52	48	—	47	53	50	50	—
CJ1W	189	51	49	—	47	53	52	48	—

(3) Opposite flanges deform equally in the riveted specimens, while the bottom attachment is the stiffer in the welded T and the top the stiffer in welded gusset plate specimens.

(4) Except in the case of *CH2W*, which had to be subjected to a modified loading program on account of previous partial failure, the deformations under normal and reversed loads are almost identical.

(5) The welded column flange stiffeners reduced the deformation of the *H* and *J* connection to 75 and 85% of their previous values, respectively.

All comparisons, heretofore, have been based on the deformation of the specimens during a complete reversal of load involving both flanges in tension

and compression and any intermediate slip that may occur. In Fig. 18 an analysis is given of the deformation that occurs during a repeated application of a normal load, within the design load limit of 70,000 lb., involving the top flange in tension and the bottom in compression and excluding non-elastic slip. As has been shown, the difference in deformation of the two flanges is due *to the difference in effect of tension and compression rather than to* structural difference.

27. Deformation of the Test Specimens, Second Series—Flexural and Shear Deflection

The deflections produced at 36 in. from the cantilever support by flexure and shear in individual regions during the *XY* load are given in Fig. 24. The total deflections are derived from the deflectometer readings and the flexural deflections from the extensometer readings according to Equations given in Art. 17. The resulting difference in deflection is assumed to be caused by shear. It is therefore obvious that, while the total deflections indicated are reliable, the component values of flexure and shear deflection are subject to the accuracy of the assumptions involved in the derivation of the former.

For example, compare the distribution of stress in Region II of the bottom attachment of the *PH* and *CH* specimens with relation to the short-extensometer gauge points, and it will be obvious that while the deformation indicated by the extensometer on the *PH* specimen will be less than the average for the cross section, owing to the concentration of stress in line with the beam welds, the extensometers on the *CH* specimens will indicate values more nearly equal to, or even greater than, the average, owing to the convergence of stress on the column web.

These errors, which occur also in the *J* specimens, will yield too great a value of $\delta\theta_{f_{\text{I}}}$ (the flexural slope developed in Region I), and similarly too small a value of $\delta\theta_{f_{\text{II+III}}}$, which in turn will yield too great a value of $\delta\Delta_{f_{\text{I}}}$ (the deflection produced by flexure in Region I) and too small a value of $\delta\Delta_{f_{\text{II+III}}}$.

The extent of such errors in flexural deflection is indeterminate and depends on the variation in the distribution of stress in Region II in the plate and column specimens. If, however, the vertical shear deformation in the column is negligible, as has been assumed, it is reasonable to explain the negative value of shear deformation obtained in Region I by the positive errors in flexural deflection recognized above. The positive values of shear deformation obtained for *CF* in Region I may be correct on account of the connection being made by rivets. An error in the flexural deflection for this region is also less probable.

Corrections to the deflections indicated, based on these assumptions, are shown as adjusted values in Fig. 24. They involve a slight decrease in the values of flexural deflection assigned to Region I and an almost identical increase in the values in Region (II + III). They do not affect the values

indicated for Region VI which therefore afford a dependable comparison of the total deformation of the connections tested during a reversal of load within the design load limits.

Similar regional values of flexural and shear deflection for a repeated normal load, up to 70,000 lb., as well as the development of these deflections during the application of the loads, were determined and plotted.

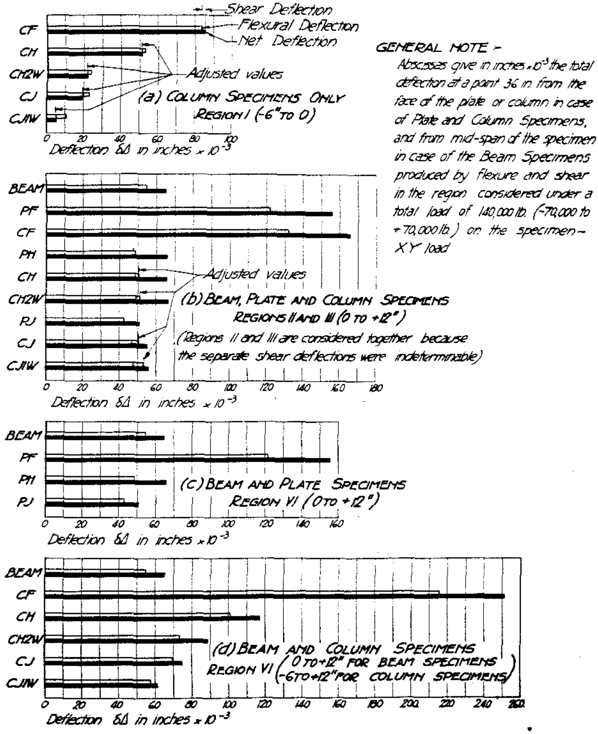


FIG. 24. *Total deflection produced at 36 in. from cantilever support by flexure and shear in individual regions due to total load change of 140,000 lb. (−70,000 to +70,000 lb.).*

A complete comparison of the performance of the specimens, including leformation during reversal within the design load and during a normal load pproaching failure, as well as the value of the failure load, is given in Fig. 25.

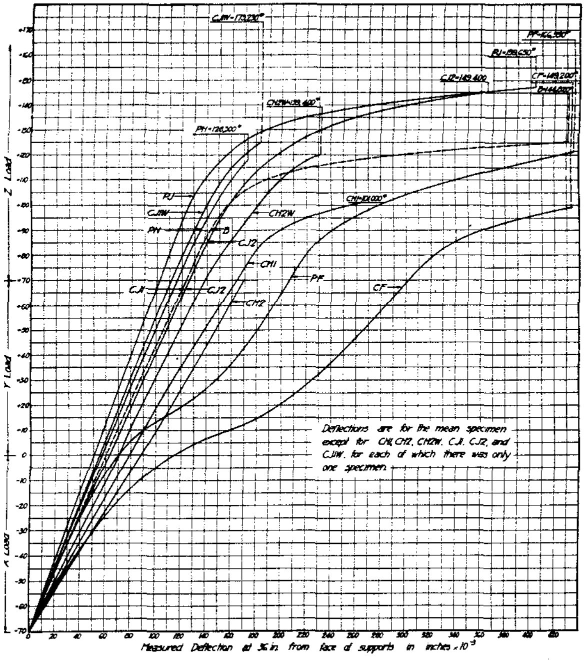


FIG. 25. *Deflection at 36 in. from cantilever support, and failure loads.*

From these results it is obvious that while both the welded types are much ›re rigid than the riveted, one of them, the welded T, is much weaker. The ıer, the gusset plate type, is somewhat stronger. The column flange ffeners greatly increase the strength and rigidity of the connections.

. *Coefficients of Restraint—First Series*

The coefficients of restraint that would be developed in the uniformly ıded, simulated beam were computed from the observed deformations of

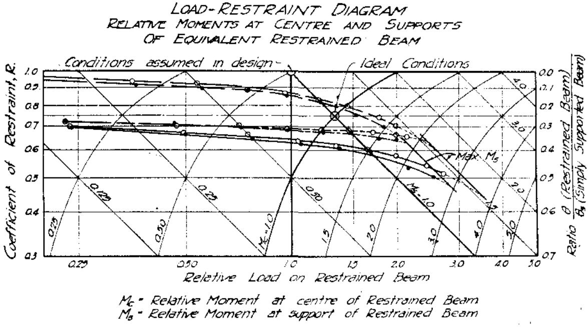
the test specimens by the method outlined in Art. 23, Section III. Determinations were made for a series of loads corresponding to loads on the double-cantilever specimens from 4,000 lb. to failure, at intervals of 4,000 lb.

Proceeding on the theoretical basis of the article mentioned, the attachments to the top and bottom flanges were compared in so far as their relative effect on the coefficient of restraint was concerned. For this purpose the slope angles θ_t and θ_b attributable to these flanges were calculated and the coefficients of restraint determined for the following three cases:

(1) Assuming that the bottom attachment developed complete restraint, or $\theta_b = 0$.

(2) Assuming that the top attachment developed complete restraint, or $\theta_t = 0$.

(3) For the combined result of the two attachments using $\theta = \theta_t + \theta_b$.



Specimen		PA1	PA2
Curves	Tension	—•—•—	—o—o—
	Compression	—•-—•—	—o-—o—
	Tension & Comp.ⁿ	—•—•—	—o—o—
Total length of 3/8" weld in two tension connections (see diagram) - in.		29	28.8
Welding time - mins.		185	145
Rate of welding - ft per hr.		7.6	9.9
Wire used (3/16" Roebling) - in		78	77
Maximum stress - lb. per lin. in.		7,340	8,340

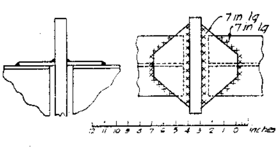
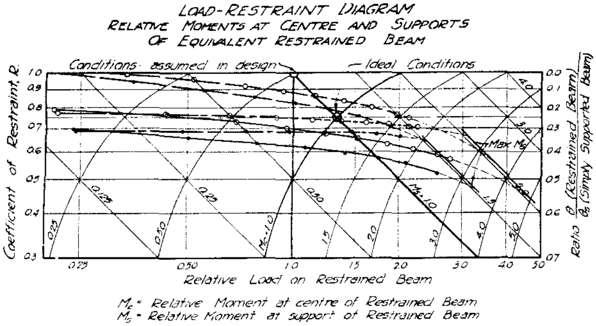


FIG. 26. *Coefficient of restraint, Connection A, first series.*

The relation of these coefficients of restraint to relative load on the simulated beam was plotted logarithmically as in Figs. 26 to 30 assuming the design load of 24,000 lb. on the beam as unity.

For each of the two specimens to which the figure pertains the coefficient of restraint that would arise if the attachment to one flange or the other



Specimen	PB1	PB2
Curves: Tension	—●— —●—	—○— —○—
Curves: Compression	—●- - -●—	—○- - -○—
Curves: Tension & Compn	—●——●—	—○——○—
Total length of 3/8" weld in two tension connections (See diagram) – in.	28	28
Welding time – mins	15	15
Rate of welding – ft per hr	9.3	9.3
Wire used (3/16" Roebling) – in.	83	84
Maximum stress – lb per lin in.	8,200	11,060

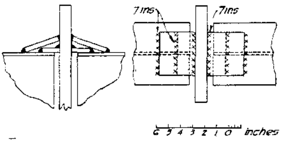
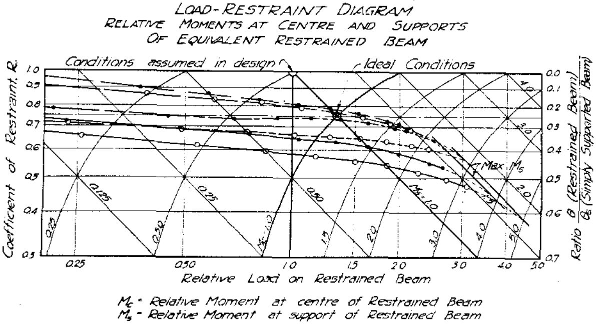


FIG. 27. *Coefficient of restraint, Connection B, first series.*



Specimen	PC1	PC2
Curves: Tension	—●— —●—	—○— —○—
Curves: Compression	—●- - -●—	—○- - -○—
Curves: Tension & Compn	—●——●—	—○——○—
Total length of 3/8" weld in two tension connections (See diagram) – in.	28	28
Welding time – mins	18	18
Rate of welding – ft per hr	7.8	7.8
Wire used (3/16" Roebling) – in.	79	78
Maximum stress – lb per lin in.	8,720	9,160

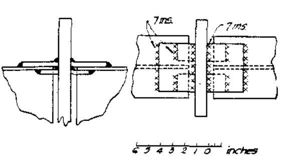
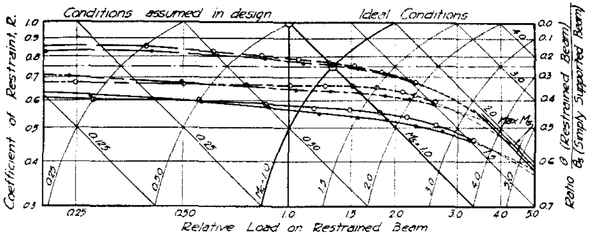


FIG. 28. *Coefficient of restraint, Connection C, first series.*

LOAD-RESTRAINT DIAGRAM
RELATIVE MOMENTS AT CENTRE AND SUPPORTS
OF EQUIVALENT RESTRAINED BEAM



M_c = Relative Moment at centre of Restrained Beam
M_s = Relative Moment at support of Restrained Beam

Specimen		PD1	PD2
Curves	Tension		
	Compression		
	Tension & Compn		
Total length of 3/8" weld in two tension connections (see diagram) in.		38	38
Welding time - mins.		22	27
Rate of welding - ft per hr		8.6	7.0
Wire used (3/16" Roebling) - in		89	110
Maximum stress - lb per lin. in		7,480	7,660

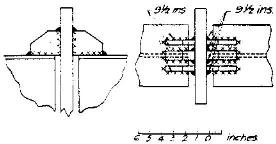
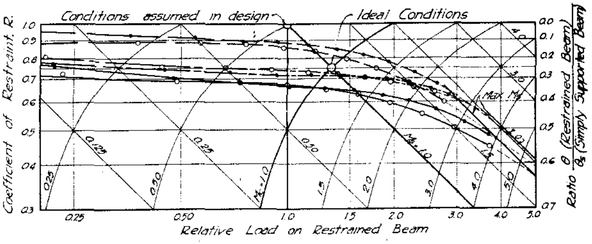


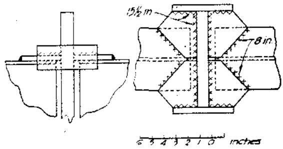
FIG. 29. *Coefficient of restraint, Connection D, first series.*

LOAD-RESTRAINT DIAGRAM
RELATIVE MOMENTS AT CENTRE AND SUPPORTS
OF EQUIVALENT RESTRAINED BEAM



M_c = Relative Moment at centre of Restrained Beam.
M_s = Relative Moment at support of Restrained Beam

Specimen		PE1	PE2
Curves	Tension		
	Compression		
	Tension & Compn		
Total length of 3/8" weld in two tension connections (see diagram) - in.		47	47
Welding time - mins		28	21
Rate of welding - ft per hr		8.4	11.2
Wire used (3/16" Roebling) - in		124	104
Maximum stress - lb per lin. in.		6,500	5,850



developed complete restraint is indicated in broken lines. The resulting curves show that the tension flange attachment is the more satisfactory of the two so far as restraint is concerned, since, if the compression attachment were perfectly rigid, the coefficient of restraint would be much higher than if the tension attachment were assumed perfectly rigid and the compression one alone deformed.

The connection as a whole failed, for this type, to develop ideal restraint, even at loads less than one-quarter of the design load. As the failure load was approached, the coefficient of restraint fell off to little more than 0.5.

For the purpose of determining the existing moment at centre or support corresponding to any combination of coefficient of restraint and beam load, one series of straight lines of equal moments at the centre and another of equal moments at the support were superimposed on the diagram. The simulated beam having been designed for perfect end restraint, the support moment for a design load of unity was itself considered to be unity. With a reduction of the coefficient of restraint to 0.5, the relative load would consequently need to be 2.0, if the support moment were to remain 1.0.

Utilizing this superimposed grid it is found by interpolation that, for example, the specimen *PA*1 developed a coefficient of restraint of 0.6 at a load 1.5 times the design load, that the support moment was about 0.9 and the centre moment about 1.4 times the design moment at the support.

While the extensometers had to be removed before failure, it is useful to consider the relation of the plotted curves of load-coefficient of restraint to the maximum moment developed in the connection. Curves of these observed moments, on the relative basis, are interpolated in Figs. 26 to 30. It is impossible to determine the coefficients of restraint at failure exactly, or the relative load on the simulated beam, which depends on coefficient of restraint, but by extending the observational curves to join the maximum moment curves at the support, an estimate may be made of them by considering the position of the intersection. If, owing to low coefficient of restraint, this point should approach the lower right hand corner of the diagram, the simulated beam would have failed at the centre before failing at the ends, as is evident from the high values indicated by the centre moment curves.

Examination of these diagrams shows that for the connections of all five types the top flange attachment is the one that promotes most the development of a relatively high coefficient of restraint. The bottom flange attachment, being more yielding, operates to offset in some measure the restraining merits of the other. For all specimens the coefficient decreases slowly as the load is increased and falls off rapidly as the load nears the failure load.

The pronounced influence of the top flange attachment in furthering a high coefficient of restraint suggests that by stiffening up the bottom flange attachment the ideal coefficient of restraint for the connection as a whole should not be difficult of attainment. Longitudinal shear deformation in the thin bottom flanges of the beams employed is believed to have been an important source of yield in the bottom attachment.

29. *Coefficients of Restraint—Second Series*

Proceeding as for the first series, the coefficients of restraint developed in the large specimens of the second series were determined for three loadings. In finding the difference between the slope angle developed in the cut-and-connected specimens and that developed in the mean plain beam, the readings of the extensometers with gauge points 18 in. from the back of the connection, or, in the case of the plain beam, 18 in. from the middle, were utilized.

Fig. 31 shows the coefficients of restraint, *R*, developed in the specimens of the second series under the *AZ* load. This loading is the initial load up to the safe design limit extended by connecting with the *Z* load, as described in

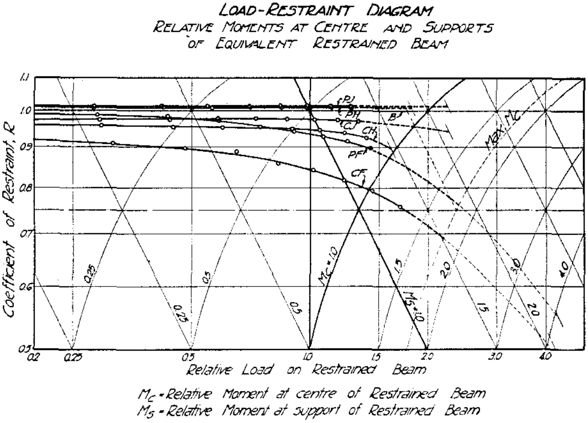


FIG. 31. *Coefficient of restraint, second series, AZ load.*

Art. 7, thus representing the probable behavior of the specimens if the initial load had been carried directly to failure. Account is thus taken of initial set, or slip, or both. Obviously, for total moment calculations, this is the correct loading to employ, as unrecoverable deformation in the connections permanently lessens their participation in the total moment developed in the span.

Coefficients of restraint greater than unity are found for *PH* and *PJ*. The riveted T specimen *CF* shows the smallest restraint, but even it, for the design load, gives a value of about 0.84.

Design of a connection for large wind moment may thus cause it to absorb an amount of gravity load moment in excess of the ideal. As illustrated in Fig. 16, restraint beyond this limit is a disadvantage and hence beams with riveted connections of the type employed in this investigation are better than those with welded connections, so far as the economical distribution of moments due to gravity loads is concerned.

A more typical situation with respect to end restraint arises when any one of the innumerable loadings following the initial load is applied. The connections have then suffered permanent deformation and permanent impairment of their restraining value. A specimen load representative of such typical loading on the simulated beam would be the Z load, but calculations of the coefficient of restraint should include a constant amount for the permanent deformation resulting between the beginning of the initial load, A, and the beginning of the Z load. Deformations due to the combined effect will be considered as arising from what has been designated the Z' load.

In the case of the riveted specimens the question arises as to how much of the non-elastic slip should be included in this combination. It is conceivable that all or none might actually occur during the application of the Z load to the simulated beam, depending on the accuracy in punching and conditions at the time of erection. For uniformity, however, the net deformation of the specimen up to the time of application of the Z load, has been used with all specimens.

Coefficients of restraint, R, for the Z' load are shown in Fig. 32. As is to be expected, the recognition of a large permanent deformation at the beginning

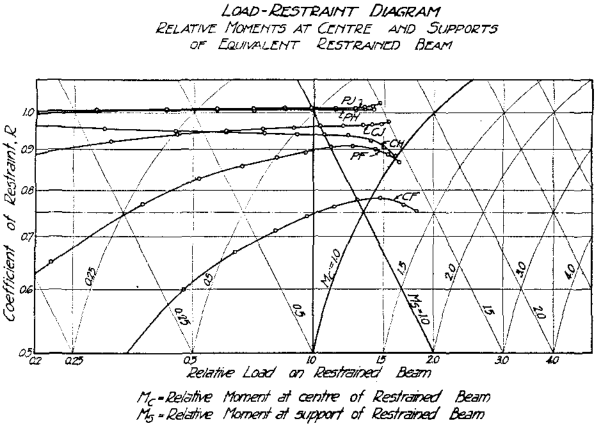


FIG. 32. *Coefficient of restraint, second series, Z′ load.*

TABLE IV

LENGTHS OF WELDS, FAILURE LOADS, STRESSES DEVELOPED AND FACTORS OF SAFETY FOR SPECIMENS OF THE FIRST SERIES

Specimen		Total nominal length of welds in complete connection, in.	Nominal length of welds that failed, in.	Effective depth, in.	Load, lb.		Estimated average ultimate stress, lb. per lin. in. of welds that failed	Factor of safety		Ratio $\frac{F_T}{F_D}$	Manner of failure
Mark	Type				Design	Ultimate		Design, F_D	Test, F_T		
BA1	Plain beam	—	—	—	24,000	63,080	—	2.50	2.63	1.05	
BA2	Plain beam	—	—	—	24,000	60,900	—	2.50	2.54	1.02	
PA1	See Fig. 1	60	7	9.7	24,000	32,550	6,700	2.02	1.36	0.67	In tension welds between connecting plates, and Plate *P*.
PA2	See Fig. 1	60	7	9.7	24,000	37,680	7,800	2.02	1.57	0.78	
PB1	See Fig. 1	60	7	10.2	24,000	36,900	7,200	2.13	1.54	0.72	
PB2	See Fig. 1	60	7	10.2	24,000	49,800	9,700	2.13	2.07	0.97	
PC1	See Fig. 1	60	7	9.5	24,000	39,230	8,300	1.98	1.63	0.82	
PC2	See Fig. 1	60	7	9.5	24,000	41,200	8,700	1.98	1.72	0.87	
PD1	See Fig. 1	70	9.5	10.4	24,000	45,700	6,500	2.94	1.90	0.65	
PD2	See Fig. 1	70	9.5	10.4	24,000	46,800	6,600	2.94	1.95	0.66	
PE1	See Fig. 1	79	8	9.4	24,000	49,180	9,200	2.24	2.05	0.92	In welds between connecting plates *X* and beam *B*, and partially in welds between plates *X* and plate *P*.
PE2	See Fig. 1	79	8	9.4	24,000	44,200	8,300	2.24	1.84	0.82	

of loading reduces the coefficients for small loads on the riveted specimens to values much below those for the *AZ* load. For the higher loads, the relative importance of the initial deformation is lessened and the coefficients become larger, even reaching the ideal value for *CF* at the design load. It is important to note that the values for *PH* and *PJ* are everywhere greater than unity, as was the case for the initial *AZ* load, while for *CH* and *CJ* they are not very much less than for the initial load.

30. *Ultimate Strength and Factors of Safety—First Series*

Ultimate strengths and factors of safety for the specimens of the first series are shown in detail in Table IV.

The design factor of safety is the estimated failure load on the specimen, based on an assumed ultimate strength in $\frac{3}{8}$-in. welds of 10,000 lb. per lineal inch, divided by the design load. The test factor of safety is, of course, the actual failure load divided by the design load, or the actual ultimate strength of welds in pounds per lineal inch divided by the working stress expressed in the same unit.

Although the design factor of safety was nominally about 2.0, it was increased in certain cases, notably for specimens *PD*1 and *PD*2, in order to compensate for anticipated uncertain behavior.

The relation of test factors of safety to design factors of safety was not so close as had been expected, since the ultimate strength of welds on test was less than the 10,000 lb. per lineal inch assumed for $\frac{3}{8}$-in. fillets. The actual realized values of the weid strength set forth in the eighth column of the table show the extent of the deficiency.

31. *Ultimate Strength and Factors of Safety—Second Series*

A general comparison of the performance of the specimens of the second series, so far as ultimate strength and factors of safety are concerned, is possible from an examination of Table V. The nature of the failures of three typical specimens *CF*1, *CH*1 and *CJ*2 is evident from Figs. 33, 34 and 35. Factors of safety were computed in the same manner as for the first series.

The relatively low design factor of safety for the plain beams, that is, 2.03, is due to the fact that the maximum flexural stress in the beam sections employed for making up the welded and riveted specimens, was assumed as occurring at 10 in. out from the face of the cantilever support. At that point, in either the plain or the cut-and-connected specimens, the factor of safety would be 2.50, but at the middle of the plain beams, which may be considered as two 54-in. cantilevers, it would be only 2.03.

While the design factor of safety originally planned for cut-and-connected specimens was from about 2.25 to 2.50, it actually varied from 1.84 for *PJ*2 to 3.15 for *PH*2. This arose either from inability to provide the exact length

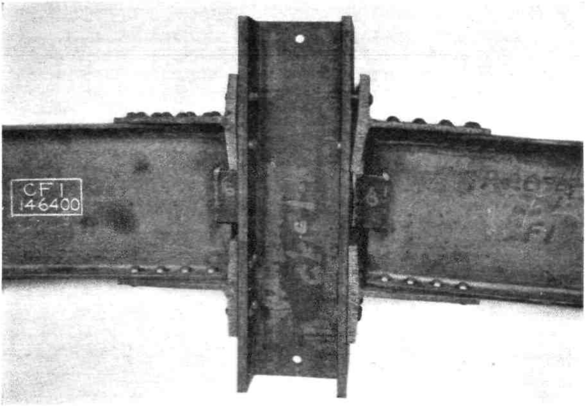


FIG. 33. *Riveted column specimen CF1 after failure at 146,000 lb.*

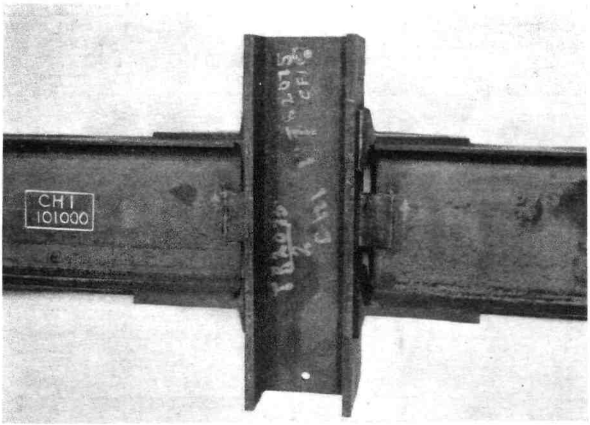


FIG. 34. *Welded column specimen CH1 after failure at 101,000 lb.*

TABLE V

LENGTHS OF WELDS, FAILURE LOADS, STRESSES DEVELOPED AND FACTORS OF SAFETY FOR SPECIMENS OF THE SECOND SERIES[1]

Specimen		Number of rivets or total nominal length of welds in complete connection[2]	Number of rivets or adjusted length of welds that failed	Effective depth, in.	Load, lb.		Estimated average ultimate stress in lb. per rivet or in lb. per lin. in., of adjusted length of welds that failed	Factor of safety		Ratio $\frac{F_T}{F_D}$	Manner of failure
Mark	Type				Design	Ultimate		Design, F_D	Test, F_T		
*BF*1	Plain beam	—	—	—	70,000	140,000	—	2.03[3]	2.00	0.99	Compression flange buckled locally
*BF*2	Plain beam	—	—	—	70,000	144,870	—	2.03[3]	2.07	1.02	Compression flange buckled locally
*BH*1	Plain beam	—	—	—	70,000	147,500	—	2.03[3]	2.11	1.04	Compression flange buckled locally
*BH*2	Plain beam	—	—	—	70,000	—	—	—	—	—	Not loaded to failure
*BJ*1	Plain beam	—	—	—	70,000	146,900	—	2.03[3]	2.10	1.04	Compression flange buckled locally
*BJ*2	Plain beam	—	—	—	70,000	—	—	—	—	—	Not loaded to failure
*PF*1	Plate specimen, riveted T's	48, ⅞-in. rivets	8, ⅞-in. rivets	18.7	70,000	165,100	29,800 lb. per rivet	2.45[4]	2.36	0.96	Rivets did not fail. Beam flanges and web buckled. Shear plate welds began to fail at 150,000 lb.
*PF*2	Plate specimens, riveted T's	48, ⅞-in. rivets	8, ⅞-in. rivets	18.7	70,000	168,000	30,100 lb. per rivet	2.45[4]	2.40	0.98	Rivets did not fail. Beam flanges buckled at end of connection
*PH*1	Plate specimen, welded T's	197 in.	30.1	18.9	70,000	139,000	6,600 lb. per in.	3.01[5]	1.99	0.66	Welds of tension T and of shear plates failed at plate face
*PH*2	Plate specimen, welded T's	197 in.	31.5	18.9	70,000	118,000	5,400 lb. per in.	3.15[5]	1.69	0.54	Welds of tension T failed at plate face

*PJ*1	Plate specimen, welded gussets	222 in.	17.3	21.6	70,000	153,300	11,100 lb. per in.	1.98[6]	2.19	1.11	Welds of tension gusset and of shear plates failed at plate face
*PJ*2	Plate specimen, welded gussets	222 in.	16.1	21.6	70,000	166,000	12,900 lb. per in.	1.84[5]	2.37	1.29	Beam flanges and web buckled. One shear plate weld failed at 100,000 lb.
*CF*1	Column specimen, riveted T's	32, ⅞-in. rivets 32, ¾-in. rivets	8, ¾-in. rivets	18.7	70,000	146,400	26,400 lb. per rivet	2.38[4]	2.09	0.88	Extreme tension rivets failed
*CF*2	Column specimen, riveted T's	32, ⅞-in. rivets 32, ¾-in. rivets	8, ¾-in. rivets	18.[illegible]	70,000	152,000	27,400 lb. per rivet	2.38[4]	2.17	0.91	Extreme tension rivets failed
*CH*1	Column specimen, welded T's	197 in.	29.4	18.9	70,000	101,000	4,900 lb. per in.	2.94[5]	1.44	0.49	Welds of tension T failed at column face
*CH*2*W*	Column specimen, welded T's, stayed flanges	326 in.	23.9	18.9	70,000	139,400	8,300 lb. per in.	2.39[5]	1.99	0.83	Final load on specimen in reversed position. Welds of tension T failed at column face[4]
*CJ*1*W*	Column specimen, welded gussets, stayed flanges	351 in.	17.6	21.6	70,000	173,230	12,300 lb. per in.	2.01[5]	2.47	1.23	Beam flanges and web buckled. Column stiffener weld failed at 159,760 lb. No failure in other welds
*CJ*2	Column specimen, welded gussets	222 in.	17.5	21.6	70,000	149,400	10,700 lb. per in.	2.00[6]	2.13	1.06	Welds of a tension gusset failed at column face

[1] *Allowance is made in the length of welds that failed for the variation of the fillet size from the desired ⅜ in. by adjusting the lengths of the fillets.*

[2] *This excludes the welds for shear plates and web stiffeners, which were common to all specimens.*

[3] *Based on assumed ultimate strength of 45,000 lb. per sq. in. in compression flange, at mid-span. At 10 in. from centre of span, the design factor of safety was 2.50.*

[4] *Based on tension rivets.*

[5] *Based on tension welds to face of support.*

[6] *Centre of extreme tension horizontal weld between T and column face began to fail at 73,000 lb., as did weld of one shear plate. Column stiffener welds failed at 120,000 lb.*

of welds, or number of rivets, required to satisfy the working stresses of the established specification, or from a deliberate increase in weld lengths to guard against too early failure, as in the cases of *PH*1 and *PH*2.

Test factors of safety bear a satisfactory relation to the design factors of safety in the case of *PF*1, *PF*2, *PJ*1, *PJ*2, *CJ*1*W* and *CJ*2.

On the other hand, the welded T-connections *PH*1, *PH*2, *CH*1 and *CH*2*W* gave poor results, by reason of the lack of uniformity of stress in the welds. For the plate specimens *PH*1 and *PH*2 early failure occurred at about the

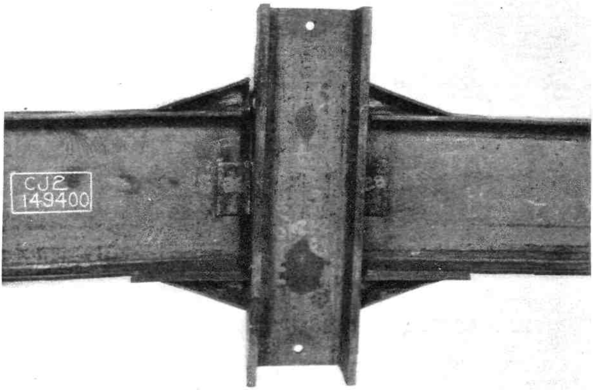


FIG. 35. *Welded column specimen CJ2 after failure at 149,000 lb.*

centre of length of the vertical welds connecting the T to the plate, while for the column specimens *CH*1 and *CH*2*W* it occurred at about the centre of the horizontal welds connecting the T to the column flange, that is, opposite the column web. The great variation in resistance to horizontal deformation afforded by the various portions of a welded connection of this type make it relatively inefficient.

The column specimens of the riveted T-type gave smaller relative factors of safety than the plate specimens of this type. This was due no doubt to a lesser uniformity of stress distribution amongst the tension rivets connecting the T to the column flange, and to the failure of the shorter grip for the $\frac{3}{4}$-in. rivets in the column flange to compensate for the use of smaller tension rivets in the column specimens.

TABLE VI

DRIFT ANGLES IN RADIANS DUE TO DEFORMATION OF CONNECTED BEAM IN 19-FT. CLEAR PANEL, AND DUE TO THE CONNECTIONS ALONE, THE END MOMENT VARYING FROM CAPACITY POSITIVE MOMENT TO CAPACITY NEGATIVE MOMENT

Case	Beam with connections indicated below								
	Plain beam integral with column	*PF*	*PH*	*PJ*	*CF*	*CH*	*CH2W*	*CJ*	*CJ1W*
Connected beam	0.00676	0.00898	0.00653	0.00641	0.01168	0.00799	0.00727	0.00717	0.00682
Plain beam integral with column	0.00676	0.00676	0.00676	0.00676	0.00676	0.00676	0.00676	0.00676	0.00676
Connections only	0	0.00222	−0.00023	−0.00035	0.00492	0.00123	0.00051	0.00041	0.00006
Drift per 12-ft. story due to connections only, in.	0	0.319	0.0331	0.0504	0.709	0.177	0.0735	0.0591	0.00864

32. *Story Drift Angle*

On the assumption that the point of inflection of the beam in the simulated panel is at mid-span, the reasonableness of which has been justified in Art. 24, the story drift angles attributable to the various connections were readily computed from the test results.

For the reversing load, XY, the total drift angle due to the cut-and-connected beam in the simulated panel is the total computed movement of the point of inflection of the beam at right angles to a normal to the column at the end of the beam, divided by the half span. The values of this deflection having been determined, the drift angles that would arise from the deformation of the 18-in., 47-lb. C.B. with its connections in the simulated bay, assuming the connections to be stressed from capacity positive moment to capacity negative moment, and the drift angles attributable to the connections alone, would be as indicated in Table VI.

Examination of the table indicates that the drift angles chargeable to the connections varied only from -0.00035 radian for PJ to $+0.00492$ for CF. The negative sign for PH and PJ shows that, owing to the reinforcement of the ends of the beam by the details, the resulting construction was stiffer than for a plain beam integrally connected to the column.

The significance of these angles is indicated in the table by the corresponding drift in inches that would occur in a 12-ft. story. The excessive drifts for the riveted T-connections are to a considerable extent due to non-elastic slip in the shear rivets connecting the stem of the T's to the beam flange.

On the assumption that a capacity wind force in one direction is applied to the building standing in a vertical position after previous loadings had brought it to seasoned or steady elastic state, the drift angles would be one-half of those listed in Table VI for capacity reversing load.

V. Summary and Conclusions

The principal findings of the investigation are briefly summarized below. Based upon the observed behavior of the specimens tested the indicated conclusions appear to be warranted for the types of connections studied.

A. *Coefficient of Restraint*

(1) At the design load the coefficient of restraint developed by the connections of the first series, designed for beam continuity only, varied from 0.57 to 0.67, as compared with an ideal value of 0.75. The coefficient increases slowly as the load decreases from the design value and decreases rapidly as the load increases therefrom. The lowest value was shown by the D connection, in which three parallel vertical plates were used for the tension attachment, and the highest by the E connection, in which both horizontal and vertical plates were utilized for the tension attachment (see Figs. 26 to 30, inclusive). Attainment of the ideal coefficient of restraint would, therefore, apparently necessitate somewhat heavier details than those adopted for this series.

(2) At the design load the coefficient of restraint developed by the connections of the second series, designed primarily for wind moment, was always equal to, or greater than, the ideal value for beam continuity, 0.75, this high value being partly due to the incidental reinforcing of the beam flanges by connection material. The smallest values were given by the riveted specimens. Whether they were riveted or welded, connections to a column flange gave smaller restraint than the corresponding connections to a column web. For any capacity gravity loading subsequent to the first one, the coefficient of restraint for connections to a column flange varied from 0.75, for a riveted T, to 0.96 for the welded gusset type; for connections to a column web, it varied from 0.90 for a riveted T to 1.01 for the two types of welded connection. Wind connections of the kind tested will develop, therefore, a higher coefficient of restraint than the most economical value required to resist gravity loads only (Fig. 32).

B. *Story Drift Due to Connections*

(3) Due to the incidental reinforcement of the flanges near its ends, the beam, when welded to a plate simulating a column web, developed less drift angle than would have been developed by a plain beam integral with the column. The drift angle for such connections was, therefore, negative. Loss of rigidity due to connections may, consequently, be entirely obviated where welded connections are made to a column web. On the other hand, T's riveted to a column web gave, for a 19-ft. clear panel, and for an end moment varying from capacity positive moment to capacity negative moment, a total, or extreme, drift angle of 0.0022 radian, or a drift of 0.32 in. for a 12-ft. story (see Table VI).

(4) T's riveted to a column flange gave, for the same panel and the same loading, a total drift angle of 0.0049 radian, or a linear drift of 0.71 in. per story. Welded T's gave 0.0012 radian and 0.18 in., while welded gusset connections gave 0.0004 radian, and 0.06 in. Supporting the column flanges by welded stays between them reduced these quantities to 0.0005 radian and 0.07 in. for the welded T's and to 0.00006 radian and 0.009 in. for the welded gusset type (see Table VI). Marked improvement in stiffness of a frame is consequently made possible by the substitution of welded for riveted connections.

(5) For a capacity wind load in one direction only the drift angles and linear drifts due to the connections tested would, if the building were plumb at the beginning of the lateral loading, be one-half of the above amounts for capacity reversing load.

C. *Sources of the Deformation*

(6) In the connections designed for beam continuity only, and subjected to load in one direction only, the top (tension) attachment was in all cases stiffer than the bottom (compression) attachment (see Figs. 26 to 30, inclusive). Transfer of the axial compression from the bottom flange by longitudinal welds at the thin edges of the flange was accompanied by considerable

yield due to horizontal shear in the flanges, which was responsible for approximately two-thirds of the loss of restraint in these specimens. The use of sections with heavier flanges would have reduced this loss and increased the values of restraint almost to those given for the tension connection alone.

(7) In the wind connections to a column web, the top and bottom attachments, under capacity reversing load, gave practically equal longitudinal deformations for all three types. In the connections to a column flange, the riveted T's gave nearly equal deformations in the two attachments; in the welded T's the top attachment gave, on an average, 20% more deformation than the bottom one; in the welded gusset type the bottom attachment gave 13% more deformation than the top one (see Table III). The influence of the half-width of the column on the end slope angle may, therefore, make itself felt to a substantially greater extent in one attachment than in the other, the more yielding attachment depending on the type of connection.

(8) In wind connections to the simulated web of a column the ratios of the flexural slope, under capacity reversing load, developed in the first 2½ in. of the connection to that developed in 12 in., the nominal length of the connection, were as follows: riveted T, 0.25; welded T, 0.42; welded gusset, 0.12 (see Table II). The flexure in the flanges of the welded T apparently contributes a considerable part of the total yield of this type of connection.

(9) In wind connections to the flange of a column, the flexural slope attributable to the half-column width under capacity reversing load bore to the total flexural slope in the nominal length of the connection plus the half-width of the column, the following ratios: riveted T, 0.35; welded T, 0.49; welded gusset, 0.29. When stiffeners were welded to the column flanges, the ratio was reduced to 0.30 and 0.16 for the welded T and the welded gusset, respectively. From a third to a half of the flexural slope in a connection to a column flange may, therefore, be charged to the deformation in the column. This may be reduced by as much as a third by the introduction of flange stiffeners (see Table II).

(10) In the wind connections to a column flange, the half-column width gave, for the welded T, 63%, and for the welded gusset, 27% of the flexural slope developed by the riveted T. When welded stiffeners were added to the column flanges, the welded T gave only 28% and the welded gusset 13%. In the first 2½ in. of the connection the welded T gave 62% and the welded gusset 17% of the flexural slope given by the riveted T. In the next 9½ in. the percentages were 27 and 42, respectively. Consequently, so far as flexural slope is concerned, and this is responsible for most of the angular deformation at a joint, the welded connections were definitely superior to the riveted ones in all regions (see Table II).

(11) The aggregate longitudinal elastic deformation in tension during the application of the reversing load was greater than the aggregate deformation in compression. For the welded specimens the excess varied from 4% for the welded gusset type with column stiffeners to 33% for the welded T-type without column stiffeners. For the riveted T's the excess was much greater,

being 56% for the connection to a column flange and 70% for the connection to a column web (see Table III).

(12) For the riveted T's a large part of the longitudinal deformation was non-elastic slip, being 31% for the connection to a column web and 46% for a connection to a column flange (see Table III).

(13) Except in the case of the welded T-connection to a column flange, which had to be subjected to a modified loading program on account of previous partial failure, the deformations under normal and reversed loads were almost identical (see Table III).

(14) The welded stiffeners to the column flanges reduced the longitudinal deformation of the welded T and the welded gusset connections to 75 and 85% of their previous values, respectively (see Table III).

D. *Ultimate Stresses and Factors of Safety*

(15) In the specimens of the first series, designed for beam continuity only, failures occurred in the welds and generally between the connecting plates and the plate simulating the web of the girder or column to which the beam would be connected. The ultimate strength of the $\frac{3}{8}$-in. fillet welds in transverse shear varied from 6500 to 9700 lb. per lineal inch, as compared with an estimated ultimate strength of 10,000 lb. per lineal inch on the basis of uniform distribution of stress (see Table IV).

(16) For the first series, the ratios of the test factor of safety to the design factor of safety, based on assumed uniform distribution of stress, was in every case below unity, varying from 0.65 to 0.97. Lack of uniformity of stress distribution was no doubt responsible for a part of the deficiency (see Table IV).

(17) In the specimens of the second series, designed primarily as wind connections, the average load on each of the $\frac{7}{8}$-in. rivets of the tension attachment to the simulated column web at the moment of failure of the compression flange of the beam was 29,950 lb., and on each of the $\frac{3}{4}$-in. rivets of the tension attachment to the column flange at the failure of these rivets it was 26,900 lb., as compared with estimated ultimate loads of 31,000 lb. and 30,000 lb., respectively, on the basis of uniform distribution of loads amongst the rivets. In the welded specimens, the stress in the adjusted length of the $\frac{3}{8}$-in. tension welds to the face of the support at failure of the specimen, which usually occurred by rupture of these welds, varied from 4900 lb. per lineal inch for one of the T-specimens to 12,900 lb. per lineal inch for one of the gusset plate specimens, as compared with an estimated ultimate strength of 10,000 lb. per lineal inch on the basis of uniform distribution of stress (see Table V).

(18) For the riveted specimens, the ratio of the test factor of safety to the design factor of safety varied from 0.88 to 0.98, while for welded T-specimens it varied from 0.49 to 0.83, and for welded gusset plate specimens, from 1.06 to 1.29. It is thus evident that non-uniformity of stress may cause the capacity of certain types of connections to fall considerably below that estim-

ated on the basis of uniform distribution. While for the welded T-type the deficiency may be as much as 50%, nevertheless, for a carefully designed gusset plate type the desired factor of safety may be readily obtained (see Table V).

Acknowledgments

The late Professor Peter Gillespie had given some thought to the problem of continuity of restraint of ordinary steel beams carrying gravity loads and, in a preliminary way, had outlined certain experimental and theoretical work. The present authors, in continuing the investigation, extended its scope and, in addition, included a study of a series of heavy wind connections. Acknowledgment is here made (in alphabetical order) of the able and conscientious services of P. A. Ballachey, H. St. Paul Bntler, A. H. Corbett, W. B. Dunbar, C. E. Helwig, G. V. Helwig, E. A. Phillips and E. A. Sudden.

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ON THE ACOUSTIC RADIATION FIELD OF THE PIEZO-ELECTRIC OSCILLATOR AND THE EFFECT OF VISCOSITY ON TRANSMISSION[1]

By Louis V. King[2]

Abstract

The radiation field due to a circular disc oscillating with uniform amplitude in a closely fitting circular aperture surrounded by an infinite rigid flange has been studied in detail, the velocity potential being expressed as an infinite integral involving cylindrical co-ordinates. The procedure adopted may be used to study the effect of viscosity on the characteristics of the radiation field. While having little effect on the field in the neighborhood of an oscillator generating waves in water, the viscosity has a marked effect on the range of distant transmission, which in consequence is appreciably affected by temperature. Of considerable practical importance is the existence of an optimum wave-length for constant output and a given distance of transmission. By means of a simple chart, it is possible to determine the optimum wave-length for a given transmitter when the level of reception for a given range is stated.

Section 1. Introduction

The piezo-electric oscillator originally due to Langevin (9) for the production of beams of sound at supersonic frequencies in water or air is now well known, and is described in easily accessible textbooks and numerous scientific papers (17, p. 147). A few features of the radiation field are well known, having been worked out in terms of polar co-ordinates by Lord Rayleigh (12, v. II, §302, p. 162).

In view of the interesting experiments by R. W. Boyle and his coworkers (5), on the exploration of the radiation field in the immediate neighborhood of the oscillating disc, the writer has developed the complete theory in terms of cylindrical wave-functions when the disc is surrounded by an infinite flange. This procedure enables the velocity potential at any point to be exactly expressed as a definite integral involving Bessel functions, so that pressure variations may be computed close to the oscillating disc as well as at a great distance. The analysis also makes it possible to take into account the viscosity of the medium and to estimate its influence on the transmission.

Section 2. Determination of the Velocity Potential in Cylindrical Co-ordinates

We consider a rigid circular disc of radius a caused to oscillate in a closely fitting circular aperture in a fixed rigid wall of infinite extent, constituting a "piston diaphragm" with an infinite flange, actuated by a suitably arranged piezo-electric (or other) mechanism.

For periodic motion of the diaphragm with frequency $f=\omega/2\pi$ we have to solve for the velocity potential ϕ from the wave equation

$$\nabla^2\phi + \kappa^2\phi = 0 \ , \tag{1}$$

where $\kappa=\omega/c$, and c is the velocity of sound in the medium.

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In terms of cylindrical co-ordinates (z, ρ) referred to the centre of the diaphragm as origin and the axis of z directed into the medium, it is easily seen that a solution of Equation (1) (2, §27, p. 73)* representing divergent waves is given by

$$\phi = \int_0^\infty e^{-\mu z} J_o(\lambda\rho) f(\lambda) \frac{\lambda}{\mu} d\lambda \ , \tag{2}$$

where $\mu = (\lambda^2 - \kappa^2)^{\frac{1}{2}}$, and $f(\lambda)$ is a function of the parameter λ to be determined by the boundary conditions over the plane $z=0$. If $\dot{x}$ is the velocity of the disc at any instant, we evidently have

$$-\left(\frac{\partial\phi}{\partial z}\right)_{z=o} = \int_0^\infty \lambda J_o(\lambda\rho) f(\lambda) d\lambda = \begin{cases} \dot{x} & (o < \rho < a) \ . \\ 0 & (a < \rho < \infty) \ . \end{cases} \tag{3}$$

The solution of Equation (3) as an integral equation is at once furnished by Hankel's inversion theorem (7, 15, 16) which gives, if the velocity $\dot{x}$ is everywhere constant over the disc,

$$f(\lambda) = \dot{x} \int_0^a \rho J_o(\lambda\rho) d\rho = \frac{\dot{x}a}{\lambda} J_1(\lambda a) \ . \tag{4}$$

We now have by substitution in Equation (2) the exact and compact expression for the velocity potential

$$\phi = a\dot{x} \int_0^\infty e^{-\mu z} J_o(\lambda\rho) J_1(\lambda a) \frac{d\lambda}{\mu} \ , \tag{5}$$

valid at all points of the field.

Section 3. Calculation of Acoustic Impedance of Diaphragm

The wave not being plane, the pressure variation, or harmonic excess pressure, at any point on the diaphragm is given by

$$\delta p_o = \rho_o(\dot{\phi})_{z=o} = \rho_o a \ddot{x} \int_0^\infty J_o(\lambda\rho) J_1(\lambda a) \frac{d\lambda}{\mu} \ ,$$

where ρ_o is the density of the medium. It will be noticed that δp_o is not constant, as would be the case for an infinite diaphragm transmitting plane waves, but is a function of ρ.

Integrating over the area of the disc, we easily find for the total pressure P_o

$$P_o = \int_0^a \delta p_o 2\pi\rho d\rho = 2\pi\rho_o a^2 \ddot{x} \int_0^\infty \frac{J_1^2(\lambda a) d\lambda}{\mu\lambda} \ . \tag{6}$$

It is at once evident that the integral of Equation (6) consists of a real and imaginary part. We write

$$M_1 - iM_2 = \int_0^\infty \frac{J_1^2(\lambda a)}{(\lambda^2 - \kappa^2)^{\frac{1}{2}}} \frac{d\lambda}{\lambda} = \int_\kappa^\infty \frac{J_1^2(\lambda a)}{(\lambda^2 - \kappa^2)^{\frac{1}{2}}} \frac{d\lambda}{\lambda} - i\int_0^\kappa \frac{J_1^2(\lambda a)}{(\kappa^2 - \lambda^2)^{\frac{1}{2}}} \frac{d\lambda}{\lambda} \ . \tag{7}$$

* *This type of cylindrical wave-function does not appear to have been used hitherto in acoustic problems, although such solutions of the wave equation are well known in electromagnetic theory, especially in connection with antenna radiation fields.*

The first integral is at once expressible in terms of Struve's function $H_1(2\kappa a)$ by writing $z=\kappa a$ and $u=\lambda a$ in the known integral (16; §13. 47(9); 7, p. 210),

$$M_1 = \int_z^\infty \frac{J_1^2(u)}{(u^2-z^2)^{\frac{1}{2}}}\,\frac{du}{u} = \frac{1}{2z^2}\,H_1(2z)\ . \tag{8}$$

This gives

$$M_1 = \int_\kappa^\infty \frac{J_1^2(\lambda a)}{(\lambda^2-\kappa^2)^{\frac{1}{2}}}\,\frac{d\lambda}{\lambda} = \frac{1}{2\kappa^2 a}H_1(2\kappa a)\ . \tag{9}$$

To calculate the second integral in Equation (7), write $\lambda=\kappa\sin\theta$. Then, writing $z=\kappa a$,

$$M_2 = \int_0^\kappa \frac{J_1^2(\lambda a)}{(\kappa^2-\lambda^2)^{\frac{1}{2}}}\,\frac{d\lambda}{\lambda} = \frac{1}{\kappa}\int_0^{\frac{1}{2}\pi}\frac{J_1^2(z\sin\theta)}{\sin\theta}\,d\theta\ . \tag{10}$$

Obviously

$$\frac{d}{dz}(\kappa M_2) = 2\int_0^{\frac{1}{2}\pi} J_1(z\sin\theta)\ .\ J'_1(z\sin\theta)d\theta = \int_0^{\frac{1}{2}\pi} J_1(z\sin\theta)\left(J_o(z\sin\theta)-J_2(z\sin\theta)\right)d\theta\ .$$

If we write $\zeta=2z\cos\phi$, we may express the products J_1J_o and J_1J_2 in the form (16: §5. 43(1) and (2)),

$$\left.\begin{aligned} J_1(z\sin\theta)\ .\ J_o(z\sin\theta) &= \frac{2}{\pi}\int_0^{\frac{1}{2}\pi}\cos\phi J_1(\zeta\sin\theta)d\phi \\ J_1(z\sin\theta)\ .\ J_2(z\sin\theta) &= -\frac{2}{\pi}\int_0^{\frac{1}{2}\pi}\cos 3\phi J_1(\zeta\sin\theta)d\phi\ . \end{aligned}\right\} \tag{11}$$

Interchanging the order of integration,

$$\frac{d}{dz}(\kappa M_2) = \frac{4}{\pi}\int_0^{\frac{1}{2}\pi}\cos\phi\cos 2\phi d\phi\int_0^{\frac{1}{2}\pi}J_1(\zeta\sin\theta)d\theta\ .$$

Since (16: §12. 11(2); §10. 42(3) and 7, p. 39),

$$\int_0^{\frac{1}{2}\pi}J_1(\zeta\sin\theta)d\theta = \left(\frac{\pi}{2\zeta}\right)^{\frac{1}{2}}H_{\frac{1}{2}}(\zeta) = \frac{1-\cos\zeta}{\zeta}\ , \tag{12}$$

we have

$$\frac{d}{dz}(\kappa M_2) = \frac{2}{\pi z}\int_0^{\frac{1}{2}\pi}\cos 2\phi\Big(1-\cos(2z\cos\phi)\Big)d\phi\ .$$

The first integral vanishes, while the second is

$$-J_2(2z) = \frac{2}{\pi}\int_0^{\frac{1}{2}\pi}\cos 2\phi\ \cos(2z\cos\phi)d\phi\ .$$

Thus

$$\frac{d}{dz}(\kappa M_2) = \frac{1}{z}J_2(2z)\ ,$$

which gives on integration

$$\kappa M_2 = \tfrac{1}{2}\left(1-\frac{J_1(2z)}{z}\right)\ ,$$

the constant of integration being chosen to make κM_2 vanish with z as it should according to Equations (9) and (10). We then have from Equations (7), (9) and (10)

$$M_1 - iM_2 = \int_0^\infty \frac{J_1^2(\lambda a)}{\mu}\frac{d\lambda}{\lambda} = \frac{H_1(2\kappa a)}{2\kappa^2 a} - \frac{i}{\kappa}\left(1 - \frac{J_1(2\kappa a)}{\kappa a}\right) . \tag{13}$$

If m_o is the mass of the diaphragm and μ_o is the natural restoring force, the equation of motion is

$$m_o\ddot{x} + \mu_o x = F - P_o ,$$

where F is the total pressure exerted by the piezo-electric drive.

Since $\ddot{x} = -\omega^2 x$ and $\dot{x} = i\omega x$, we have, introducing the value of P_o from Equations (6) and (13),

$$\ddot{x}\left(m_o + \frac{\pi\rho_o a}{\kappa^2}H_1(2\kappa a)\right) + \dot{x}\left(1 - \frac{J_1(2\kappa a)}{\kappa a}\right)\pi\rho_o a^2 c + \mu_o x = F , \tag{14}$$

as the equation of motion, in agreement with that deduced by Lord Rayleigh (12, v. 2, p. 169, Eq. 29). If we denote by m_a the *acoustic mass* and by k_a the *acoustic damping* or radiation constant, the equation of motion of the diaphragm is, in general, of the form

$$(m_o + m_a)\ddot{x} + k_a\dot{x} + \mu_o x = F , \tag{15}$$

and, in the present instance, we have for a piston diaphragm,

$$m_a = \frac{\pi\rho_o a}{\kappa^2}H_1(2\kappa a) \qquad \text{and} \qquad k_a = \pi\rho_o a^2 c\left(1 - \frac{J_1(2\kappa a)}{\kappa a}\right) . \tag{16}$$

In particular, we derive m_a and k_a for diaphragms of linear dimensions small and large compared to the wave-length λ :

Small diaphragms ($\kappa a = 2\pi a/\lambda \ll 1$).

From well known formulas (16: §10.4) we find,

$$H_1(2\kappa a) \sim \frac{2}{3}\frac{(2\kappa a)^2}{\pi} + \ldots\ldots , \qquad 1 - J_1(2\kappa a)/\kappa a \sim \tfrac{1}{2}\kappa^2 a^2 + \ldots\ldots$$

so that

$$m_a \sim \tfrac{8}{3}\rho_o a^3 \qquad \text{and} \qquad k_a \sim \tfrac{1}{2}\rho_o\pi a^4\omega^2/c . \tag{17}$$

Large diaphragms ($\kappa a = 2\pi a/\lambda \gg 1$).

For large values of $2\kappa a$ we make use of the asymptotic expansion (16; § 10.4),

$$H_1(2\kappa a) \sim 2/\pi + \ldots\ldots + Y_1(2\kappa a) ,$$

so that, neglecting terms of order $(2\kappa a)^{-\frac{1}{2}}$ and smaller, we have, approximately

$$\left.\begin{aligned} m_a &\sim 2a\rho_o/\kappa^2 = a\rho_o\lambda^2/(2\pi^2) . \\ k_a &\sim \pi a^2\rho_o c . \end{aligned}\right\} \tag{18}$$

Section 4. Calculation of Radiation Output

It follows from Equations (6) and (7) that we may write the total pressure P_o on the diaphragm in the form

$$P_o = 2\pi a^2\rho_o\ddot{x}(M_1 - iM_2) . \tag{19}$$

In terms of the velocity amplitude $|\dot{x}|$ of the diaphragm, we have

$$\dot{x} = |\dot{x}|e^{i\omega t} \quad \text{and} \quad \ddot{x} = i\omega|\dot{x}|e^{i\omega t} .$$

At any instant the rate at which the diaphragm is doing work by compressing the medium is, expressing both P_o and $\dot{x}$ as real quantities,

$$dW/dt = P_o\dot{x} = 2\pi a^2\rho_o\omega|\dot{x}|^2(M_1 \sin \omega t + M_2 \cos \omega t) \cos \omega t .$$

On taking the time average, we have for the rate of output of radiation

$$[dW/dt] = \pi a^2\rho_o\omega|\dot{x}|^2M_2 = \tfrac{1}{2}k_a|\dot{x}|^2 = \tfrac{1}{2}\pi a^2\rho_o c\,[1-J_1(2\kappa a)/\kappa a] , \tag{20}$$

where k_a is the acoustic radiation constant defined by Equation (15).

Numerical Example.

To obtain a numerical estimate of the physical quantities involved in a piezo-electric oscillator in this and following sections, we consider a disc of radius $a=30$ cm. to be radiating 50 watts on a wave-length of $\lambda=3.77$ cm. in sea water of density $\rho_o=1.025$, the velocity of sound being taken as $c=1.490 \times 10^5$ cm. per sec., so that $\rho_o c=1.53 \times 10^5$.

The area of the disc is $\pi a^2=2826$ cm^2., so that the sound is propagated at the rate of 0.0177 watts per cm^2.

Corresponding to the wave-length $\lambda=3.77$ cm., we have the frequency $f=4 \times 10^4$ and $\omega=2\pi f=2.50 \times 10^5$, while $\kappa a=2\pi a/\lambda=50$.

In these circumstances it is sufficiently accurate to employ the approximation from Equation (20),

$$[dW/dt] \sim \tfrac{1}{2}\pi a^2\rho_o c|\dot{x}|^2 = 50 \times 10^7 \text{ ergs/sec} . \tag{21}$$

Inserting numerical values we find for the velocity amplitude, $|\dot{x}|=1.53$ cm./sec., while the corresponding displacement amplitude is 612×10^{-8} cm. $=612$ Angstrom units, about one tenth the wave-length of sodium light, or roughly, 300 times the diameter of a hydrogen molecule.

Section 5. Discussion of the Radiation Field, General Theorems

The expression for the velocity potential deduced in Equation (5) of Section 2,

$$\phi = a\dot{x}\int_0^\infty e^{-\mu z}J_o(\lambda\rho)J_1(\lambda a)\,\frac{d\lambda}{\mu} \tag{22}$$

is, as far as the writer is aware, new. It is easily seen that we may write this in the form $\phi=a\dot{x}(P-iQ)$, where

$$\left.\begin{aligned} P &= \int_0^\infty J_o(\kappa\rho\cosh\phi) \,.\, J_1(\kappa a\cosh\phi) \,.\, e^{-\kappa z\sinh\phi}\,d\phi \\ Q &= \int_0^{\frac{1}{2}\pi} J_o(\kappa\rho\sin\theta) \,.\, J_1(\kappa a\sin\theta) \,.\, e^{-\kappa z\cos\theta}\,d\theta . \end{aligned}\right\} \tag{23}$$

The pressure amplitude is obviously given by,

$$|\delta p| = \rho_o|\dot{\phi}| = a\rho_o\kappa c|\dot{x}|\{P^2+Q^2\}^{\frac{1}{2}} \tag{24}$$

Mapping of the radiation field is best carried out in cylindrical co-ordinates in terms of the integrals P and Q computed by graphical methods, or preferably by means of a mechanical integrator.

In general, the expression of integrals of the type of Equation (22) in terms of known functions of the polar co-ordinates (r, θ) is not a simple matter. It is, however, possible to obtain an asymptotic expression for the radiation field at distances great compared to the wave-length and linear dimensions of the wave source, corresponding to a velocity potential of the form

$$\phi = \int_o^\infty e^{-\mu z} J_o(\lambda\rho) f(\lambda) \frac{\lambda d\lambda}{\mu} . \tag{25}$$

We will suppose that $f(\lambda)$ is capable of expansion as a convergent power series in μ^2, *i.e.*,

$$f(\lambda) = a_o + a_2\mu^2 + a_4\mu^4 + \ldots + a_{2n}\mu^{2n} + \ldots = \sum_o^\infty a_{2n}\mu^{2n} .$$

Since

$$(d/dz)^{2n} e^{-\mu z} = \mu^{2n} e^{-\mu z} ,$$

we at once see that

$$\int_o^\infty e^{-\mu z} J_o(\lambda\rho) f(\lambda) \frac{\lambda d\lambda}{\mu} = \sum_{n=o}^{\infty} a_{2n} \left(\frac{\partial}{\partial z}\right)^{2n} \int_o^\infty e^{-\mu z} J_o(\lambda\rho) \frac{\lambda d\lambda}{\mu} = \sum_{n=o}^{\infty} a_{2n} \left(\frac{\partial}{\partial z}\right)^{2n} \frac{e^{-ikr}}{r} ,$$

where we have made use of the well known integral (16: §13.47 (4)),

$$\int_o^\infty e^{-\mu z} J_o(\lambda\rho) \frac{\lambda}{\mu} d\lambda = \frac{e^{-ikr}}{r} , \quad r^2 = (\rho^2 + z^2)^{\frac{1}{2}} \tag{26}$$

which, with the time factor $e^{i\omega t}$ represents a diverging wave.

Since

$$\partial/\partial z = \cos\theta\partial/\partial r - (\sin\theta/r)\partial/\partial\theta ,$$

we have the symbolic representation of the integral in the form

$$\int_o^\infty e^{-\mu z} J_o(\lambda\rho) f(\lambda) \frac{\lambda d\lambda}{\mu} = f\left(\kappa^2 + \cos^2\theta \left(\frac{\partial}{\partial r}\right)^2\right)^{\frac{1}{2}} \cdot \frac{e^{-ikr}}{r} . \tag{27}$$

Since

$$F\left(\frac{\partial}{\partial r}\right) \cdot \frac{e^{-ikr}}{r} = e^{-ikr} F\left(\frac{\partial}{\partial r} - i\kappa\right) \frac{1}{r} ,$$

we may write the right-hand side of Equation (27) in the expanded form

$$\int_o^\infty e^{-\mu z} J_o(\lambda\rho) f(\lambda) \frac{\lambda d\lambda}{\mu} = e^{-ikr} \left[\sum_n a_{2n} \left(\frac{\partial}{\partial r} - i\kappa\right)^{2n} \cos^{2n}\theta \right] \frac{1}{r} = \frac{e^{-ikr}}{r} \sum_n \frac{A_{2s}(\cos\theta)}{r^{2s}} , \tag{28}$$

where $A_{2s}(\cos\theta)$ are polynominals in $\cos^2\theta$.

We easily find

$$A_o = \sum_n (-1)^n a_{2n} (\kappa\cos\theta)^{2n} = f(\kappa^2 - \kappa^2\cos^2\theta) = f(\kappa\sin\theta) .$$

We thus have for the asymptotic expansion of the radiation field,

$$\phi = \int_o^\infty e^{-\mu z} J_o(\lambda\rho) f(\lambda) \frac{\lambda}{\mu} d\lambda \sim \frac{e^{-ikr}}{r} f(\kappa\sin\theta) . \tag{29}$$

This theorem is apparently new and is especially useful as it enables us to calculate the rate of radiation of energy in any particular direction. In general $f(\lambda)$ will be complex, so that writing $f(\kappa \sin\theta)=f_1(\theta)+if_2(\theta)$, we have taking the real part of Equation (29),

$$\phi \sim f_1(\theta)\frac{\cos(\omega t-\kappa r)}{r} - f_2(\theta)\frac{\sin(\omega t-\kappa r)}{r} \quad ,$$

from which it follows, on taking a time average, that for unit spherical area in direction θ, the rate of radiation of energy, or the radiation intensity, $I(\theta)$, is

$$I(\theta) = \left[\frac{dw}{dt}\right]_\theta \sim \tfrac{1}{2}\frac{\kappa^2 c\rho_o}{r^2}\left[f_1^2(\theta)+f_2^2(\theta)\right] \quad , \tag{30}$$

and therefore the rate of radiation in a cone of semi-vertical angle θ is,

$$\left[\frac{dW}{dt}\right]_o^\theta \sim \pi\kappa^2 c\rho_o \int_o^\theta \left[f_1^2(\theta)+f_2^2(\theta)\right]\sin\theta d\theta \quad . \tag{31}$$

Section 6. Applications to Supersonic Oscillator

If we now apply the theorems of the last section to the integral expression of Equation (22) for the velocity potential of a supersonic oscillator, we at once obtain for the intensity in a direction θ the well known expression

$$I(\theta) \sim \tfrac{1}{2}c\rho_o|\dot{x}|^2\frac{a^2}{r^2}\frac{J_1^2(\kappa a\sin\theta)}{\sin^2\theta} \quad , \tag{32}$$

while, according to Equation (31),

$$\left[\frac{dW}{dt}\right]_o^\theta \sim \pi c\rho_o a^2|\dot{x}|^2\int_o^\theta \frac{J_1^2(\kappa a\sin\theta)}{\sin\theta}d\theta \quad . \tag{33}$$

We notice that if we write $\theta=\frac{1}{2}\pi$ in Equation (33) we obtain the same result as that given in Equation (20) for the radiation output computed at the diaphragm.

We notice from Equation (32) that the polar curve for intensity shows a series of loops bounded by cones in which the intensity vanishes to the order a^2/r^2. These angles are given by $\kappa a\sin\theta_s=j_{1s}$, where j_{1s} is the s'th root of $J_1(x)=0$.

The maxima are given by $\kappa a\sin\theta_s=j_{2s}$ where j_{2s} is the s'th root of $J_2(x)=0$.

Applying these results to the numerical example of Section 4 for which $\kappa a=50$, we find for the directions of minimum and maximum intensities:

θ_{min}		4°.9	8°.1	11°.7	etc.
θ_{max}	0°	5°.9	9°.7	12°.8	etc.

while

$I(\theta_{max})/I(o)$	$=1$	.0175	.00416	.00160	etc.

Of considerable interest is a numerical estimate of the relative proportion of energy radiated in the first loop. The integral of Equation (33) as it stands

is not easily exactly evaluated in finite terms. When θ is small, we notice that with an error of less than 1% for an angle of 4°.9 in the numerical example quoted,

$$\int_o^{\theta_1} \frac{J_1^2(\kappa a \sin\theta)}{\sin\theta}\, d\theta > \int_o^{\theta_1} \frac{J_1^2(\kappa a \sin\theta)}{\sin\theta} \cos\theta\, d\theta = \tfrac{1}{2}\left[1 - J_o^2(\kappa a \sin\theta_1) - J_1^2(\kappa a \sin\theta_1)\right] .$$

Since $J_1(\kappa a \sin\theta_1) = 0$ at the first maximum, we have for the proportion of power radiated in the first loop to the total the ratio,

$$\frac{[dW/dt]_o^{\theta_1}}{[dW/dt]_o^{\frac{1}{2}\pi}} > \frac{1 - J_o^2(\kappa a \sin\theta_1)}{1 - J_1(2\pi a)/(\kappa a)} . \tag{34}$$

This result shows that more than 84% of the radiant energy is contained in the primary beam for which $\theta_1 = 4°.9$.

Section 7. Distribution of Intensity on the Axis

If we write $\rho = 0$ in Equation (22), we obtain for the velocity potential on the axis the well known formula

$$\phi = a\dot{x}\int_o^\infty e^{-\mu z} J_1(\lambda a)\frac{d\lambda}{\mu} = \frac{\dot{x}}{i\kappa}\left[e^{-i\kappa z} - e^{-i\kappa(a^2+z^2)^{\frac{1}{2}}}\right] . \tag{35}$$

The integration is effected by multiplying each side of Equation (26) by ρ and integrating from 0 to a.

From Equation (35) we at once obtain for the pressure amplitude, writing $R^2 = a^2 + z^2$,

$$|\delta p|^2 = 4(c\rho_o)^2|\dot{x}|^2 \sin^2 \tfrac{1}{2}\kappa(R - z) . \tag{36}$$

If, as usual, we define intensity as rate of transmission of energy across unit area we have for points on the axis

$$I(0) = (c\rho_o)^2|\dot{x}|^2\,[1 + z/R]\sin^2\tfrac{1}{2}\kappa(R - z) . \tag{37}$$

The velocity-amplitude is given by

$$|q|^2 = \left[\frac{\partial\phi}{\partial z}\right]^2 = |\dot{x}|^2\left[\left(1 - \frac{z}{R}\right)^2 + \frac{4z}{R}\sin^2\tfrac{1}{2}\kappa(R - z)\right] . \tag{38}$$

The experimental interpretation of a quasi-stationary field, such as that of a piezo-electric oscillator, is seen from the last three formulas to depend on the type of apparatus employed for measuring and detecting pressure and velocity variations in the medium. An optical method which responds to changes of refractive index arising from changes of density will give fluctuations of the measured quantity according to Equation (36). A small listening probe which absorbs all the energy incident on it will give rise to fluctuations represented by Equation (37), while a small Rayleigh disc will respond to Equation (38). In these three cases the position and amplitudes of the maxima and minima will be different.

These peculiar features of a quasi-stationary field, as contrasted with the plane progressive or stationary fields, indicate the precautions to be observed in interpreting such experiments as those of Boyle and his coworkers (6). The experimental results referred to are not strictly comparable with the

theoretical calculations of this paper owing to the absence of the infinite flange which makes a rigorous mathematical solution possible. Furthermore a practical disc shows variations of amplitude not contemplated by theory. The acoustic radiation pressure on a circular disc even of circumference small compared with the wave-length may be calculated, and it is found, again, that the variation of the readings is again different from any of the formulas quoted. Only when z/a is very large or very small, so that the waves are practically plane, are the results of observation by different methods easily comparable.

We notice that Equations (36) and (37) agree in giving rise to a finite number of points of zero pressure amplitude or intensity. These are given by the roots of $\sin \frac{1}{2}\kappa(R-z) = o$, *i.e.*, for $\frac{1}{2}\kappa(R-z) = s\pi$, where s takes integral values.

In terms of the wave-length, the position of these silent spots is given by

$$2z = \frac{a^2}{s\lambda} - s\lambda \ .$$

Since z must be positive, it is readily seen that the number of silent spots possible is equal to the number of integers less than a/λ.

In the numerical example already quoted for which $a = 30$ cm. and $\lambda = 3.77$ cm. ($\kappa a = 50$), there are seven silent spots situated at the following distances from the plate: 3.9, 6.7, 14.5, 24.4, 35.3, 56.2 and 118 cm.

A theoretical investigation by the author (not yet published) shows that only with pressure discs of circumference very small compared to the wave-length can these silent spots be observed by this method. The writer is informed by Dr. Boyle, however, that by means of a "listener" of diameter of the same order as a wave-length the "silent spots" (or probably marked minima of intensity) can be detected (6: pp. 183-187).

Section 8. Calculation of Velocity Potential and Pressure over the Diaphragm

At the centre of the diaphragm we have, writing $z = 0$ in Equation (35)

$$\phi_o = \dot{x}a \int_0^\infty J_1(\lambda a) \frac{d\lambda}{\mu} = \frac{\dot{x}}{i\kappa}(1 - e^{-i\kappa a}) \ , \tag{39}$$

in agreement with the result obtained by N. W. McLachlan (11). From this we obtain for the pressure amplitude at the centre, due to the reaction of the medium,

$$|\delta p_o| = 2c\rho_o|\dot{x}| \sin \tfrac{1}{2}\kappa a \ . \tag{40}$$

Considering the numerical example of Section 4, we have

$$c\rho_o = 1.53 \times 10^5 \qquad \text{and} \qquad |\dot{x}| = 1.53 \text{ cm./sec.}$$

giving

$$|\delta p_o| = 0.47 \times 10^6 \sin\tfrac{1}{2}\kappa a \ \text{ dynes/cm}^2.$$

indicating that for an average emission of 0.0177 watts per cm^2. from a disc of 30 cm. radius the pressure amplitude may amount to nearly half an atmosphere.

At the edge of the diaphragm $\rho = a$, so that

$$\phi_a = \dot{x}a\int_0^\infty J_o(\lambda a)J_1(\lambda a)\frac{d\lambda}{\mu} = \frac{\dot{x}}{2i\kappa}\Big[\Big(1-J_o(2\kappa a)\Big) + iH_o(2\kappa a)\Big] \quad , \tag{41}$$

also in agreement with the result obtained by McLachlan (11). The integral is easily obtained by noticing that

$$J_o(\lambda a)J_1(\lambda a) = \frac{2}{\pi}\int_0^{\frac{1}{2}\pi} J_1(2\lambda a\cos\theta)\cos\theta d\theta \quad , \tag{42}$$

and then utilizing Equation (39) with a replaced by $2a\cos\theta$.

The pressure amplitude at the edge is seen to be

$$|\delta p_a| = \tfrac{1}{2}\rho_o c|\dot{x}|\Big[\Big(1-J_o(2\kappa a)\Big)^2 + H_o^2(2\kappa a)\Big]^{\frac{1}{2}}.$$

In the numerical example just cited with $\kappa a = 50$, we have as a rough approximation $|\delta p_a| \backsim \frac{1}{2}\rho_o c|\dot{x}|$, amounting to one-quarter of the maximum possible pressure at the centre of the diaphragm.

Of considerable interest is the variation of pressure over the diaphragm. The calculation is, however, a matter of some difficulty. We note that

$$J_1(\lambda\rho)J_1(\lambda a) = \frac{1}{\pi}\int_0^\pi \cos\theta\, J_o(\lambda R)d\theta \quad , \tag{43}$$

where

$$R^2 = a^2 + \rho^2 - 2a\rho\cos\theta \quad . \tag{44}$$

Utilizing the well known integral of Equation (26), we have

$$\int_0^\infty \frac{\lambda}{\mu} J_1(\lambda\rho)J_1(\lambda a)d\lambda = \frac{1}{\pi}\int_0^\pi \frac{e^{-i\kappa R}}{R}\cos\theta\, d\theta \quad . \tag{45}$$

In terms of the spherical wave-functions $\psi_n(x)$ and $f_n(x)$ defined in terms of Bessel functions by the relations (8, p. 504),

$$\left.\begin{array}{ll} x^n\psi_n(x) = (\frac{1}{2}\pi/x)^{\frac{1}{2}} J_{n+\frac{1}{2}}(x) & x^n\phi_n(x) = (-1)^n(\frac{1}{2}\pi/x)^{\frac{1}{2}} J_{-n-\frac{1}{2}}(x) \\ x^n f_n(x) = -i(\frac{1}{2}\pi/x)^{\frac{1}{2}} H^{(2)}{}_{n+\frac{1}{2}}(x) & f_n(x) = \phi_n(x) - i\psi_n(x) \end{array}\right\} \tag{46}$$

we have the well known expansion, valid for $0 < \rho < a$ (8, p. 512),

$$\frac{e^{-i\kappa R}}{R} = \sum_{s=o}^\infty (2s+1)\,(\kappa a)^s f_s(\kappa a)(\kappa\rho)^s\psi_s(\kappa\rho)P_s(\cos\theta) \quad . \tag{47}$$

Denoting

$$A_s = \frac{1}{\pi}\int_0^\pi \cos\theta\, P_s(\cos\theta)d\theta = \frac{1}{\pi}\int_{-1}^{+1}\cot\theta\, P_s(\cos\theta)d(\cos\theta) \quad , \tag{48}$$

we easily deduce from the known expansion of $\cot\theta$ in spherical harmonics,

$$A_{2s} = 0 \text{ and } A_{2s+1} = \frac{2s+2}{2s+1}\left[\frac{1}{2}\cdot\frac{3}{4}\cdot\ldots\cdot\frac{2s+1}{2s+2}\right]^2 \tag{49}$$

If now we integrate both sides of Equation (45) with respect to ρ from 0 to a, we finally obtain after a few reductions,

$$\phi_{z=o} = \phi_o - \frac{a\dot{x}}{\kappa}\sum_{s=o}^\infty (4s+3)A_{2s+1}(\kappa a)^{2s+1}f_{2s+1}(\kappa a)\int_0^{\kappa\rho}\zeta^{2s+1}\psi_{2s+1}(\zeta)d\zeta \quad , \qquad (\rho < a) \quad , \tag{50}$$

where ϕ_o is the potential at the centre given by Equation (39). We have for $s=o$,

$$\int_o^{\kappa\rho} \zeta\psi_o(\zeta)d\zeta = 1 - \psi_o(\kappa\rho) = 1 - \frac{\sin \kappa\rho}{\kappa\rho} \ . \tag{51}$$

Denoting

$$I_n = \int \zeta^n\psi_n(\zeta)d\zeta \ , \tag{52}$$

it is a simple matter to derive the recurrence formula, useful in numerical computations,

$$(n+1)I_{n+1} = nI_{n-1} - (2n+1)\zeta^n\psi_n(\zeta) \ , \tag{53}$$

which is also valid when $\psi_n(\zeta)$ is replaced by $f_n(\zeta)$ or $\phi_n(\zeta)$ in Equations (52) and (53).

From Equation (50) we may easily obtain the pressure amplitude by resolving the series into real and imaginary parts.

When $a<\rho<\infty$, the appropriate expansion, Equation (47), is obtained by interchanging a and ρ. In this case we integrate both sides of Equation (45) between a and ∞, and finally obtain

$$\phi_{z=o} = -\frac{a\dot{x}}{\kappa}\sum_{s=o}^{\infty}(4s+3)A_{2s+1}(\kappa a)^{2s+1}\psi_{2s+1}(\kappa a)\int_{\infty}^{\kappa\rho}\zeta^{2s+1}f_{2s+1}(\zeta)d\zeta, \quad (\rho>a) \ . \tag{54}$$

For $s=o$, we have

$$\int_{\infty}^{\kappa\rho} \zeta f_1(\zeta)d\zeta = -f_o(\kappa\rho) = -e^{-ik\rho}/\rho \ , \tag{55}$$

while the integrals may, in general, be computed by a recurrence formula of the type of Equation (53).

It does not appear possible to express the velocity potential ϕ for any point of the radiation field in terms of spherical wave-functions, although an attempt to do so has recently been made by Backhaus (1) and Ruedy (13), based on the assumption that for $r>a$ an expansion of the type

$$\phi_o = \sum A_s(\kappa r)^s f_s(\kappa r)P_s(\cos\theta) \tag{56}$$

is valid as representing divergent waves. It is possible to determine the coefficients A_s to make the value of ϕ on the axis, for which $\theta=0$, agree with the known value, Equation (35). The writer has verified by a simple independent calculation the values of the coefficients A_s obtained by Backhaus (1). Since the velocity potential is everywhere finite within the hemisphere $r=a$, we may similarly represent the velocity potential within this radius by

$$\phi_i = \sum B_s(\kappa r)^s\psi_s(\kappa r)P_s(\cos\theta) \ . \tag{57}$$

The writer's method is equally adapted to the determination of the coefficients B_s to give the required value, Equation (35), on the axis. It is found, however, that at $r=a$ the values of ϕ_o and ϕ_i do not agree for a given value of θ. There is similarly a discontinuity in the values of $\partial\phi_o/\partial r$ and $\partial\phi_i/\partial r$ at $r=a$, while the values of ϕ_i and ϕ_o for $\theta=\frac{1}{2}\pi$ do not agree with the expansions of Equations (50) and (54).

In fact, continuity of pressure and velocity over the sphere $r = a$ would require, if expansions of the type of Equations (56) and (57) were valid,

$$\left.\begin{aligned} A_s f_s(\alpha) &= B_s \psi_s(\alpha) \ , \\ A_s \frac{d}{d\alpha}\Big[\alpha^s f_s(\alpha)\Big] &= B_s \frac{d}{d\alpha}\Big[\alpha^s \psi_s(\alpha)\Big] \end{aligned}\right\} , \tag{58}$$

where we have written $\alpha = \kappa a$.

Since $f_s'(\alpha) = -\alpha f_{s+1}(\alpha)$ and similarly for $\psi_s'(\alpha)$, we have on differentiating the second and utilizing the first,

$$A_s f_{s+1}(\alpha) = B_s \psi_{s+1}(\alpha) \ . \tag{59}$$

Remembering that

$$f_s(\alpha) = \phi_s(\alpha) - i\psi_s(\alpha) \ ,$$

we would have, on eliminating A_s and B_s ,

$$f_{s+1}(\alpha)\psi_s(\alpha) - f_s(\alpha)\psi_{s+1}(\alpha) = 0$$

or

$$\phi_{s+1}\psi_s - \psi_{s+1}\phi_s = 0 \ , \tag{60}$$

which is clearly impossible, since (8, p. 505),

$$\phi_{s+1}\psi_s - \psi_{s+1}\phi_s = \alpha^{-(2s+3)} \ . \tag{61}$$

We are thus obliged to conclude that the velocity potential due to a rigid circular disc oscillating within an infinite flange cannot rigorously be expressed as a series of the type of Equation (56). When $\alpha = \kappa a$ is large, the discrepancy between Equations (60) and (61) is at most of the order $(\kappa a)^{-3}$, so that Backhaus' (1) formulas, although not rigorous, are not seriously in error, since his computations for comparison with experimental results are carried out for $(\kappa a) = 5, 8, 17$.

Section 9. Distance of Audible Transmission in a Frictionless Medium

On the axis $\theta = 0$, Equations (32) and (34) agree in giving for the intensity, when κa is large,

$$I(0) = \tfrac{1}{8}\rho_o c\,|\dot{x}|^2 a^2 \frac{(\kappa a)^2}{r^2} = \frac{1}{4\pi}\frac{(\kappa a)^2}{r^2}\left[\frac{dW}{dt}\right] \ , \tag{62}$$

where $[dW/dt]$ is the total radiation output given by Equation (20).

If we estimate easily audible reception on the axis to be represented by a level of 30 decibels, this corresponds to $I(0) = I_o \times 10^3$, where I_o is the "threshold intensity", $I_o = 10^{-16}$ watt per cm². We thus have, to estimate the distance of easily audible reception,

$$\frac{1}{4\pi}\frac{(\kappa a)^2}{r^2}\left[\frac{dW}{dt}\right] = 10^{-13} \text{ watts/cm}^2 . \tag{63}$$

If we utilize the numerical data already quoted for a piezo-electric 50-watt oscillator for which $\kappa a = 50$, we find for the distance of easily audible transmission about 3200 km. The range of piezo-electric transmitters which have been built and tested hardly exceeds 20 km. It is evident that under actual conditions of use in the sea, many factors operate to reduce this theoretical estimate. Among these we may mention the generation of elastic waves in

the oscillating disc and surrounding flange, the scattering and diffraction of the sound waves by the free surface of the sea and the inhomogeneities of temperature and density, the effect of turbulence due to surface waves, and finally the effect of viscosity, which we proceed to examine in the next section.

Section 10. On the Effect of Viscosity on the Radiation Field of a Supersonic Oscillator

We denote by μ the coefficient of viscosity and by ν the kinematic coefficient of viscosity given by

$$\nu = \mu/\rho_0 \ . \tag{64}$$

It is well known that when viscosity is taken into account, the general equations of periodic sound waves are satisfied by the velocity components

$$u = -\frac{\partial\phi}{\partial x} \qquad v = -\frac{\partial\phi}{\partial y} \qquad w = -\frac{\partial\phi}{\partial z} \ , \tag{65}$$

where ϕ satisfies the modified wave equation (8, §361, p. 654),

$$(\nabla^2 + \kappa^2)\phi = 0 \ , \tag{66}$$

and κ is given by

$$\kappa^2 = \frac{\omega^2}{c^2 + \frac{4}{3}i\nu\omega} \ . \tag{67}$$

In all practical cases relating to supersonic waves in water, $\frac{4}{3}\nu\omega/c^2$ is of the order 10^{-6}, so that denoting $\kappa_0 = \omega/c$ we may, with sufficient accuracy write

$$\kappa \sim \kappa_0 - i\kappa', \qquad \text{where} \qquad \kappa' = \tfrac{2}{3}\nu\kappa_0^2/c \ . \tag{68}$$

In a perfect fluid, the solution we have obtained exhibits a tangential discontinuity of velocity, or slip, at the boundary of the flange and diaphragm communicating vibrations to the medium. In an actual fluid no such discontinuity can exist. The tangential motion close to the vibrating surface is modified, and may even be reversed in direction. It is well known, however (8, §361, p. 657), that this effect extends only to a stratum whose thickness is of the order

$$\beta^{-1} = (2\nu/\omega)^{\frac{1}{2}} \ . \tag{69}$$

For water, $\beta^{-1} = .055/f^{\frac{1}{2}}$ cm., where f is the frequency. The motion at a distance from the boundary which is a moderate multiple of β^{-1} will be practically irrational, with a velocity potential given by Equation (44).

It is evident that in the supersonic range of frequencies, at a distance of the order β^{-1} from the diaphragm or infinite flange, the motion conforms practically to the same boundary conditions as those obtaining in the ideal problem of the frictionless medium, as long as the diameter of the diaphragm is also very great compared to β^{-1}.

The fundamental solution, Equation (5), of the modified wave-equation (66) is also valid for complex values of κ, and care has been taken to make certain that all formulas derived from it are also valid for a complex κ. It follows that with the restrictions referred to above, we have available the characteristics of the supersonic radiation field as affected by the viscosity of the medium.

Section 11. On the Effect of Viscosity on the Axial Distribution of Intensity

If we write $\kappa=\kappa_o-i\kappa'$ in Equation (35), we easily obtain for the pressure amplitude on the axis

$$|\delta p|^2 = \frac{4(\rho_o c)^2\,|\dot{x}|^2}{|\kappa/\kappa_o|^2}\,e^{-\kappa'(R+z)}\,[\sinh^2 \tfrac{1}{2}\kappa'(R-z) + \sin^2 \tfrac{1}{2}\kappa_o(R-z)\,] \quad . \tag{70}$$

We now see that theoretically, owing to the effect of viscosity, there are no absolutely silent spots on the axis. It is of some interest, however, to evaluate the minimum pressure amplitude in the practical example already quoted for which $\kappa_o a=50$, $a=30$ cm., $\lambda=3.77$ cm. and $|\dot{x}|=1.53$ cm./sec.

At 20° C. we may take for sea water $\mu_{20}=.0101$, while $\rho_o c=1.53\times 10^5$. We then find from Equation (68),

$$\kappa' = \tfrac{2}{3}\mu\kappa_o^2/(c\rho_o) = 1.20\times 10^{-7} \quad .$$

It is obvious that with this extremely small value of κ', minima of $|\delta p|^2$ occur very nearly at the same points as the "silent spots" of the ideal theory, *i.e.*, for $\frac{1}{2}\kappa_o(R-z)=s\pi$.

We see from Equation (70) that at these minima the pressure amplitude is, approximately,

$$|\delta p|_{min} \sim 2(\rho_o c)|\dot{x}|s\pi(\kappa'/\kappa_o) \quad . \tag{71}$$

The minimum nearest to the diaphragm corresponds to $s=7$, at 3.9 cm. distance.

From Equation (71) we easily obtain $|\delta p|_{min}=0.74$ dynes/cm²., while the ratio

$$|\delta p_{min}|/|\delta p_{max}| \sim s\pi(\kappa'/\kappa_o) = 1.6\times 10^{-6} \quad .$$

A pressure amplitude of 0.74 dynes/cm². in a plane progressive wave corresponds to an intensity

$$I = \tfrac{1}{2}|\delta p|^2/(\rho_o c) = 1.81\times 10^{-6}\ \text{ergs/cm}^2 \quad .$$

equivalent to 32.5 decibels, while the pressure maxima in the radiation field are easily seen from Equation (70) to be practically unaffected by viscosity and correspond in a plane wave to 148 decibels.

As already mentioned in Section 6, the interpretation of observations of the field characteristics on the axis depends on the type of receiver employed to give a measurable physical effect: notwithstanding this feature, the above calculation indicates that the effect of viscosity is sufficient to give a detectable minimum of pressure amplitude, instead of a zero minimum as required in the ideal problem of a frictionless fluid.

Section 12. On the Effect of Viscosity on the Radiation Output

The procedure of Section 4 is applicable to the problem, since the integrals M_1 and M_2 of Section 3 are valid for complex values of κ. If we confine ourselves to the case $|\kappa a|\gg 1$, we find

$$M_1 - iM_2 \sim \frac{1}{\pi\kappa^2 a} - \frac{i}{2\kappa} \quad .$$

Writing $\kappa=\kappa_o-i\kappa'$, we obtain

$$M_2 \sim \frac{1}{2\kappa_o}\left[1-\frac{4}{\pi}\left(\frac{\kappa'}{\kappa_o}\right)^2\right] \; ,$$

and finally as in Section 4,

$$\left[\frac{dW}{dt}\right] = \tfrac{1}{2}\pi\rho_o a^2 c|\dot{x}|^2 \left[1-\frac{4}{\pi}\left(\frac{\kappa'}{\kappa_o}\right)\right] \; . \tag{72}$$

In most practical cases the ratio κ'/κ_o is so small that the effect of viscosity on the radiation output as calculated for a frictionless medium is negligible.

Section 13. On the Effect of Viscosity on the Distant Radiation Field

Corresponding to a radiation field represented by the velocity potential

$$\phi = \int_o^\infty e^{-\mu z} J_o(\lambda\rho) f(\lambda) \frac{\lambda}{\mu} d\lambda \; , \tag{73}$$

the asymptotic formula (Equation 29),

$$\phi \sim f(\kappa \sin\theta) e^{-i\kappa r}/r \tag{74}$$

is easily seen to be valid for complex values of $\kappa=\kappa_o-i\kappa'$.

Writing $f(\kappa\sin\theta)=f_1(\theta)+if_2(\theta)$, we find, taking the real part of Equation (74), that

$$\phi \sim f_1(\theta)e^{-\kappa' r}\frac{\cos(\omega t-\kappa_o r)}{r} + f_2(\theta)e^{-\kappa' r}\frac{\sin(\omega t-\kappa_o r)}{r} \; , \tag{75}$$

from which we easily deduce that the intensity in a direction θ is given by

$$I(\theta) \sim \tfrac{1}{2}\rho_o c\kappa_o^2 \frac{e^{-2\kappa' r}}{r^2}\left[f_1^2(\theta)+f_2^2(\theta)\right] \; . \tag{76}$$

In particular, for the supersonic oscillator with an infinite flange, we have

$$f(\lambda) = a|\dot{x}|\frac{J_1(\lambda a)}{\lambda} \text{ and } f'(\lambda) = -|\dot{x}|\frac{a^2}{\lambda}J_2(\lambda a) \; .$$

Since κ'/κ_o is always extremely small, we have, approximately,

$$f(\kappa\sin\theta) = f_1(\theta)+if_2(\theta) \sim f(\kappa_o\sin\theta) - i\kappa'\sin\theta f'(\kappa_o\sin\theta) \; ,$$

and hence

$$I(\theta) \sim \tfrac{1}{2}\rho_o c a^2|\dot{x}|^2\frac{e^{-2\kappa' r}}{r^2}\left[\frac{J_1^2(a\kappa_o\sin\theta)}{\sin^2\theta} + (a\kappa')^2 J_2^2(a\kappa_o\sin\theta)\right] \; , \tag{77}$$

a result which expresses the influence of viscosity on the radiation field.

Section 14. Numerical Applications. Effect of Temperature on Range of Transmission

From physical tables (14, p. 163), we find for the specific viscosity of a solution of sodium chloride of density 1.024 the value 1.0107. For pure water at 0° and 20° C. we have $\mu_o=.0179$ and $\mu_{20}=.0100$.

In the absence of direct numerical data on viscosity of sea water, we may take for the coefficients of viscosity at 0° and 20° C., $\mu_o=.0181$ and $\mu_{20}=.0101$.

For the oscillator of 30 cm. radius for which $\kappa a = 50$, we find for κ' given by

$$\kappa' = \frac{2}{3}\frac{\nu}{c}\kappa_o^2 = \frac{2}{3}\frac{\mu\kappa_o}{\rho_o c}$$

at temperatures 0° and 20° C. ($\lambda = 3.77$ cm.),

$$\kappa_o' = 2.16 \times 10^{-7} \text{ and } \kappa'_{20} = 1.20 \times 10^{-7} \ . \tag{78}$$

We now notice that with these numerical values, the effect of viscosity is inappreciable in Equation (72). Furthermore, it is evident from Equation (77) that except for the exponential factor, important in cutting down transmission at extreme distances, the effect of viscosity is negligible on the angular distribution of intensity which, to less than one part in a million, is the same as that in a frictionless medium.

Of great practical importance, however, is the effect of viscosity in reducing the range of transmission. On the axis, utilizing Equation (72), we may with sufficient accuracy write Equation (77) in the form

$$I(0) \sim \frac{e^{-2\kappa' r}}{r^2} \frac{(\kappa_o a)^2}{4\pi} \left[\frac{dW}{dt}\right] \ . \tag{79}$$

To obtain a numerical estimate of the effect of viscosity, and hence of temperature, on transmission, we assume a reception level of 30 decibels, so that $I(0) = 10^{-13}$ watts per cm². With an input of 50 watts, Equation (79) requires us to solve for r from the equation

$$re^{\kappa' r} = (\kappa_o a) \times 6.3 \times 10^6 \text{ cm.}$$

It is a simple matter to compute the data shown in Table I.

TABLE I

CALCULATED EFFECT OF TEMPERATURE ON SUPERSONIC TRANSMISSION IN SEA WATER

λ, cm.	T = 0° C., μ_o = .0181			T = 20° C., μ_{20} = .0101		
	$\kappa' \times 10^6$	$\kappa' r$	r. km.	$\kappa' \times 10^6$	$\kappa' r$	r. km.
0.24	55.2	12.2	2.2	30.7	9.68	3.1
.47	13.8	8.34	6.0	7.68	7.81	10.0
.94	3.45	6.51	19.0	1.92	6.00	31
1.89	.864	4.73	54.5	.48	4.26	89
3.77	.216	3.09	143	.12	2.65	221

NOTE: *Radius of transmitter, 30 cm.; input, 50 watts; reception, 30 decibels.*

These results are shown graphically in Fig. 1, and indicate clearly the very marked effect of temperature on transmission, making evident that the effect of temperature on viscosity is able to explain in large measure the marked difference between reception of supersonic signals actually observed in the sea in summer and winter (17, p. 335 and 10).

Observations by P. Biquard (4) seem to indicate for plane stationary waves in liquids a much higher coefficient of absorption at extremely high frequencies than can be accounted for by viscosity. Thus denoting by α the observed coefficient, Biquard's (4) observations give the data shown in Table II.

TABLE II

DATA COMPUTED FROM BIQUARD (4)

Temp., °C.	f	λ, cm.	α	κ' (calcd.)
14	7.55×10^6	0.0194	1.74×10^{-3}	0.55×10^{-3}
17	7.97×10^6	0.0184	2.0×10^{-3}	0.60×10^{-3}

The last column shows that the coefficient κ' computed from the theoretical expression, Equation (67), is much smaller for these extremely high frequencies than the value α actually observed. The effect of thermal conductivity can easily be shown to be negligible, as it increases κ' only by a quantity of the order of 1.13×10^{-6} (3).

In view of the practical importance of viscosity in transmission of supersonics, it is important that the effective viscosity of sea water over the range of wave-lengths 0.5 to 5 cm. be measured, if possible, for a divergent radiation field such as that of a supersonic sound oscillator. Until such data have been obtained the numerical results of this section and the next can be regarded as having only a provisional value indicating the order of magnitude of effects which can be attributed to viscosity.

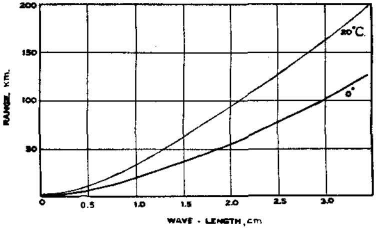

FIG. 1. *Calculated effect of temperature on supersonic transmission. Radius of transmitter, 30 cm. Input, 50 watts. Reception, 30 decibels. Viscosity of sea water: 0° C.,* $\mu_0 = 0.0181$; *20° C.,* $\mu_{20} = 0.0100$.

In this connection it will be noticed that Equation (71) gives a possible means of determining κ' experimentally, should it be possible to measure the ratio of successive minima and maxima of the pressure amplitude on the axis. The effect would be best observed as acoustic radiation pressure on a small disc, for which an appropriate formula would have to be specially worked out. It must be remembered, however, that Equation (71) is valid only for an ideal piezo-electric oscillator surrounded by a theoretically infinite rigid flange.

Section 15. Conditions for Optimum Transmission and Reception*

On referring to Equation (79) for the intensity on the axis, we notice that for constant input the intensity at a distance r is a maximum for a certain

* *Since this paper was communicated, the writer has been informed by Dr. R. W. Boyle that he has been aware of the existence of an optimum wave-length, influenced by temperature, since 1918, having arrived at similar conclusions from theoretical considerations as well as from actual observations at sea.*

wave-length. It is evident that diminishing wave-lengths narrow the angle of the primary beam and increase the factor $(\kappa_o a)$, while through a corresponding increase of κ', the exponential factor in Equation (79) is diminished. Taking κ_o as the independent variable, we easily see that $I(0)$ is a maximum for $r\kappa_o \frac{d\kappa'}{d\kappa_o} = 1$, or, since $\kappa' = \frac{2}{3}\frac{\nu}{c}\kappa_o^2$, the condition of optimum reception is

$$\frac{4}{3}\frac{\nu}{c}\kappa_o^2 r = 1 \, , \qquad \text{giving} \qquad \lambda = 4\pi\left(\frac{\nu r}{3c}\right)^{\frac{1}{2}} \; . \tag{80}$$

Since the above condition also gives $2\kappa' r = 1$, the corresponding intensity on the axis is given by

$$I(0)_{max} = \frac{a^2}{4\pi e} \cdot \frac{3c}{4\nu} \cdot \frac{1}{r^3}\left[\frac{dW}{dt}\right] \; . \tag{81}$$

It is here assumed that the electrical system actuating the transmitter can be regulated to constant acoustic output. In general, however, $[dW/dt]$ will be a known function of the frequency or wave-length under specified conditions of electrical input, and the conditions for optimum reception will have to be modified accordingly.

Numerical Example

Utilizing numerical data pertaining to a disc of 30 cm. radius and 50 watts output, we have $a = 30$ cm., $c = 1.49 \times 10^5$ cm. per sec., $e = 2.72$, $[dW/dt] = 50$ watts. At 0° C., utilizing the usual value for the viscosity, we find $\nu_o = 0.0181$, and expressing the distance of transmission by R in kilometres, we find

$$I(0)_{max} = (8.13 \times 10^{-6})/R^3 \; , \quad (T = 0^\circ \text{ C.}) \tag{82}$$

If $\alpha(0)_{max}$ expresses the intensity on the *decibel scale*, we have

$$\alpha(0)_{max} = 10 \log_{10} [I_{o\,max} \times 10^{16}] = [109.1 - 30 \log_{10} R \text{ (km.)}] \text{ decibels}, \quad (T = 0^\circ \text{ C.}) \tag{83}$$

while the corresponding wave-length is

$$\lambda(\text{cm.}) = 0.800\sqrt{R(\text{km.})} \; .$$

At 20° C. we take $\nu_{20} = 0.0101$, and the corresponding formulas are

$$\left.\begin{aligned} I(0)_{max} &= (14.57 \times 10^{-6})/R^3 \\ \alpha(0)_{max} &= [111.6 - 30 \log_{10} R(\text{km.})] \text{ decibels}, \quad (T = 20^\circ \text{ C.}) \\ \lambda \text{ (cm.)} &= 0.597\sqrt{R(\text{km.})} \end{aligned}\right\} \tag{84}$$

The above formulas are most conveniently expressed in the form of a graph plotted on semi-logarithmic paper, as shown in Fig. 2. The relations (83) and (84) connecting the level of reception in decibels with the range in kilometres give straight lines corresponding to sea water at 0° C. and 20° C., while the optimum wave-length for any given range is given by the corresponding curves and the scale on the right-hand side. For instance, at 20° C., 30 decibels reception is obtained at 550 km. for the optimum wave-length $\lambda = 13.6$ cm., whereas reference to Fig. 1 shows that the same level of

reception on a 2-cm. wave-length is only 95 km. At 0° C., 30 decibels reception is obtained at 440 km. for optimum wave-length $\lambda = 16.5$ cm., which may be compared to 55 km. on a 2-cm. wave.

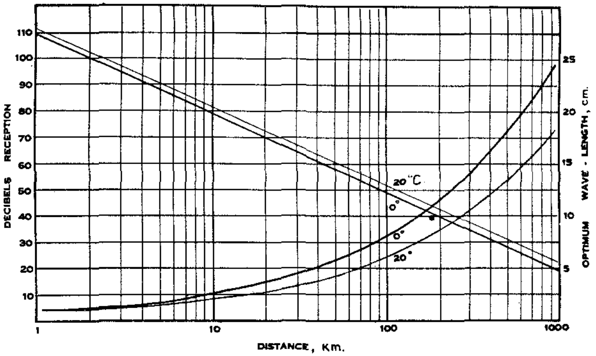

FIG. 2. *Chart for optimum reception. Input, 50 watts. Radius of transmitter, 30 cm.*

The numerical results of this section as well as the curves of Fig. 2 cannot be regarded as having more than a very rough accuracy in view of a considerable uncertainty as to viscosity of sea water at the frequencies considered, as well as to the existence of many factors affecting transmission not taken into account in the theory of the ideal transmitter.

Section 16. Summary and Conclusions

1. By the use of cylindrical wave-functions, it is possible by the application of Hankel's inversion formula to express the velocity potential due to a disc of radius a oscillating with uniform amplitude x in a closely fitting circular aperture surrounded by an infinite rigid flange in the form

$$\phi = a\dot{x}\int_0^\infty e^{-\mu z} J_0(\lambda\rho) J_1(\lambda a)\frac{d\lambda}{\mu}, \qquad \text{(i)}$$

where

$$\mu = (\lambda^2 - \kappa^2)^{\frac{1}{2}}.$$

2. The acoustic impedance of the disc as derived from the above formula agrees with the expression obtained by Lord Rayleigh's method.

3. The method of the present paper enables a large variety of radiation fields to be calculated corresponding to any given law of variation of amplitude of the disc with radius, provided always that it is surrounded by an infinite rigid flange.

4. The asymptotic expression for the distant field is shown to be

$$\phi = \int_0^\infty e^{-\mu z} J_0(\lambda \rho) f(\lambda) \frac{\lambda}{\mu} d\lambda \sim f(\kappa \sin\theta) \frac{e^{-i\kappa r}}{r} , \qquad \text{(ii)}$$

where $f(\lambda)$ may be determined when the amplitude variation over the disc is given as a function of the radius.

5. The distribution of intensity on the axis for a disc vibrating with constant amplitude is easily obtained from the velocity potential (i) and agrees with the formula obtained by Lord Rayleigh's method. The position of "silent spots" is indicated and their position worked out in a numerical example.

6. The pressure amplitude over the disc and flange is worked out, and may be expressed as an infinite series of spherical wave-functions.

7. It is shown that it is not possible, in general, to expand the velocity potential (i) as a series of spherical wave-functions involving the polar co-ordinates (r,θ).

8. The effect of viscosity on the radiation field is to replace κ in Equation (i) by the complex value $\kappa = \kappa_o - i\kappa'$, where $\kappa' = \frac{2}{3}\kappa_o^2(\nu/c)$, and ν is the kinematic coefficient of viscosity.

9. The effect of viscosity is to replace "silent spots" on the axis by minima at approximately the same positions. Numerical estimates are given of the pressure amplitude at these positions of minima in water.

10. With the usual coefficients of viscosity for water, it is shown that the effect on the radiation output and the angular distribution of the radiation field at a distance differs imperceptibly from the values for a perfect fluid.

11. Viscosity has, however, a marked effect on the range of distant transmission and, in consequence, the latter is markedly affected by temperature as illustrated by an appropriate graph.

12. Of considerable practical importance is the existence of an optimum wave-length for constant output and a given distance of transmission. By means of a simple chart it is possible to determine the optimum wave-length for a given transmitter when the level of reception (expressed in decibels) for a given range is stated.

13. It is probable in view of the results of Biquard (4) that, at supersonic frequencies, considerably higher values for the coefficient of viscosity must be taken. It is important that extended and accurate values be determined for sea water over the large range of frequencies used in practice.

14. Throughout the paper numerical estimates of various factors relating to the radiation field are calculated for a disc of 30 cm. radius having an output of 50 watts.

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ON THE THEORY OF THE X-RAY DETERMINATION OF ELASTIC SCATTERING OF CATHODE RAYS[1]

BY DAROL K. FROMAN[2]

Abstract

This paper is a continuation of Bubb's (1) theoretical work which relates the energies and momenta of cathode rays to the properties of X-ray photons produced by them. Bubb's results are used to determine a scattering function for the cathode rays in a target in terms of the intensities of X-rays of minimum wave-length emergent from the target at various angles to the incident cathode-ray stream. It is shown that the self-consistency of Bubb's results can be tested by measurements of the intensities of two components of these X-rays polarized in mutually perpendicular planes. Mention is made of an experiment now in progress designed to test Bubb's theory by an absolute measurement using a gaseous instead of a solid target.

Introduction

Bubb (1) assumes that when an electron impinges on an atom and produces a quantum of white X-radiation, the energy and momentum are conserved, and the impulse given to the atom is in the direction of the electric vector of the electromagnetic wave associated with the X-ray photon. These assumptions, among others, lead to two results of interest here: (i) that if an electron gives up its entire energy to form an X-ray photon, that photon must emerge at a calculable angle to the momentum vector of the electron before collision; (ii) that the electric vector of the X-ray produced in this case must lie in the plane containing the emergent ray and the momentum vector of the electron before collision. A direct test of these results requires the use of a target so thin that the number of electrons scattered through appreciable angles in passing through it is negligible. In Section III, below, preliminary experiments with effectively thin vapor targets are described.

If, by means of the first-mentioned result, an angular distribution of the momenta of the electrons in a thick target which would produce any observed intensity function of the X-rays of minimum wave-length is found, the state of polarization of the X-rays can be predicted from the second result. An agreement between this prediction and experiment would constitute strong evidence for both the validity of Bubb's theory and the correctness of the scattering function.

I. The Scattering Function Determined from the X-Ray Intensities

In Fig. 1 let cathode rays moving in the positive direction of the x-axis be incident on a thick target whose face is parallel to the yz-plane. Let OB represent the direction of emission of an X-ray photon of minimum wave-length. In this Section, and in the next, only those cases in which the momentum of the electron is zero after emission, thus producing rays of

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minimum wave-length, are to be considered. Let OB lie in the xy-plane making angle ϕ with the x-axis. Let OC represent the momentum of the electron producing this photon. Then by Bubb's result OC must make an angle ω with OB where ω is given by

$$\cos\omega = (1-\sqrt{1-\beta^2})/\beta \tag{1}$$

where βc is the velocity of the electron before collision and c is the velocity of light. Let Ψ be the angle through which this electron has been deflected, without loss of energy, from its original direction of motion. Let (x_1, y_1, z_1) be any point on OC and $(x_2, y_2, 0)$ be its projection on OB, and let r be the distance from 0 to (x_1, y_1, z_1). Let η be the angle between the lines joining $(x_2, y_2, 0)$ to $(x_1, y_1, 0)$ and to (x_1, y_1, z_1). Let $p(\Psi)$ be the probability that an electron incident along the x-axis be moving in a unit solid angle at an angle Ψ to the x-axis on emission of its total energy. Let $I(\phi)$ be the intensity of X-rays of wave-lengths between λ_0 and $\lambda_0+\delta\lambda$ (corresponding to electrons moving between ω and $\omega+\delta\omega$ to OB) emergent along OB. Then since the total radiation is incoherent,

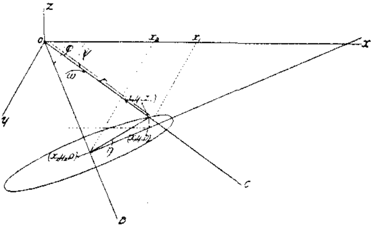

FIG. 1. *The angles between the directions of motion of electrons and X-ray photons.*

$$I_\phi = K_1 \sin\omega\delta\omega . \, 2\int_0^\pi p(\Psi)d\eta \tag{2}$$

where K_1 is an unknown constant dependent on the number of incident electrons, etc. Since ω and $\delta\omega$ are independent of η and ϕ, let $K_2 = 2K_1 \sin\omega\delta\omega$. Then,

$$I_\phi = K_2 \int_0^\pi p(\Psi)d\eta \ . \tag{3}$$

From the geometry of Fig. 1,

$$x_1 = r\cos\Psi \ ,$$

$$x_2 = r\cos\omega\cos\phi \ ,$$

and

$$x_1 - x_2 = r\sin\omega\cos\eta\sin\phi \ ,$$

Hence

$$\cos\Psi - \cos\omega\cos\phi = \sin\omega\cos\eta\sin\phi$$

or

$$\cos\eta = (\cos\Psi - \cos\omega\cos\phi)/\sin\omega\sin\phi \ . \tag{4}$$

For brevity let:

$$b = \cos\omega\cos\phi \ , \quad a = \sin\omega\sin\phi \ , \text{ and } \xi = \cos\Psi \ . \tag{5}$$

Then

$$\cos \eta = (\xi - b)/a \tag{6}$$

$$\sin \eta = \sqrt{1-\cos^2\eta} = (1/a)\sqrt{a^2-b^2+2b\xi-\xi^2} \ , \tag{7}$$

and, from Equation (4),

$$-\sin \eta d\eta = -(1/a) \sin \Psi d\Psi = d\xi/a \ . \tag{8}$$

Therefore

$$d\eta = -d\xi/a \sin \eta = -d\xi/\sqrt{a^2-b^2+2b\xi-\xi^2} \ . \tag{8a}$$

If the target is homogeneous p will be symmetrical about the x-axis and can be expressed as a function of $\cos \Psi$ $(=\xi)$. We assume it to be sufficiently general to assume a power series in ξ for p with the coefficients of all the negative powers zero. Then

$$p = \sum_{m=0}^{\infty} A_m \xi^m \tag{9}$$

where the A_m's are constant. Then by Equations (6), (8) and (9), Equation (3) becomes

$$I = -K_2 \sum_{m=0}^{\infty} A_m \int_{b+a}^{b-a} \frac{\xi^m d\xi}{\sqrt{a^2-b^2+2b\xi-\xi^2}} = -K_2 \sum_{m=0}^{\infty} A_m t_m \tag{10}$$

where (2, pp. 23-26)

$$t_m = \int_{b+a}^{b-a} \frac{\xi^m d\xi}{\sqrt{a^2-b^2+2b\xi-\xi^2}} = \left[\frac{\xi^{m-1}\sqrt{a^2-b^2+2b\xi-\xi^2}}{-m} + \frac{(2m-1)b}{m} \int \frac{\xi^{m-1}d\xi}{\sqrt{a^2-b^2+2b\xi-\xi^2}} \right.$$

$$\left. + \frac{(m-1)(a^2-b^2)}{m} \int \frac{\xi^{m-2}d\xi}{\sqrt{a^2-b^2+2b\xi-\xi^2}} \right]_{b+a}^{b-a} = \frac{(2m-1)b}{m} t_{m-1} + \frac{(m-1)(a^2-b^2)}{m} t_{m-2} \ . \tag{11}$$

Now

$$t_0 = \int_{b+a}^{b-a} \frac{d\xi}{\sqrt{a^2-b^2+2b\xi-\xi^2}} = \left[-\frac{1}{\sqrt{1}} \sin^{-1} \frac{2b-2\xi}{\sqrt{4b^2+4(a^2-b^2)}} \right]_{b+a}^{b-a} = \pm\pi = \text{a constant.} \tag{12}$$

Also,

$$t_1 = \int_{b+a}^{b-a} \frac{\xi d\xi}{\sqrt{a^2-b^2+2b\xi-\xi^2}} = \left[\frac{\sqrt{a^2-b^2+2b\xi-\xi^2}}{-1} + b\int \frac{d\xi}{\sqrt{a^2-b^2+2b\xi-\xi^2}} \right]_{b+a}^{b-a} = bt_0 \ . \tag{13}$$

Hence t_0 is a constant factor in every term of the series for I. If we let $K = -K_2 t_0$ and $T_m = \frac{t_m}{t_0}$, Equation (10) becomes

$$I = K \sum_{m=0}^{\infty} A_m T_m$$

where

$$T_0 = 1, \quad t_1 = b = \cos \omega \cos \phi \ ,$$

and

$$T_m = \frac{(2m-1)}{m} \cos \omega \cos \phi \ T_{m-1} + \frac{(m-1)}{m} (\sin^2\omega \sin^2\phi - \cos^2\omega \cos^2\phi) \ T_{m-2} \ . \tag{14}$$

We have also the normalizing condition expressing the existence of the electron:

$$1 = \int_0^\pi p 2\pi \sin \Psi d\Psi \ . \tag{15}$$

Substituting in Equation (15) from Equations (5), (8) and (9),

$$1 = -2\pi \int_1^{-1} \sum_{m=0}^{\infty} A_m \xi^m d\xi$$

$$= 2\pi \sum_{m=0}^{\infty} \left[A_m \frac{\xi^{m+1}}{m+1} \right]_{-1}^{1}$$

$$= 4\pi \sum_{n=0}^{\infty} \frac{A_{2n}}{2n+1} \quad \text{where } n \text{ is integral.}$$

Therefore

$$K = 4\pi \sum_{n=0}^{\infty} \frac{KA_{2n}}{2n+1} \ . \tag{16}$$

Now if a sufficient number of the coefficients, KA_m, of T_m in Equation (14) be found by determinants to fit any experimentally determined $I(\phi)$, Equation (16) can be used to find K and thus to find A_m. Knowing A_m, $p(\Psi)$ can be computed directly from Equations (9) and (5). T_m decreases rapidly with increasing m, especially for high velocity cathode rays, and in view of the work of Wagner (3) and Webster (4) it is thought that $I(\phi)$ may be fitted within the limits of error of reasonably accurate intensity measurements using but the first few terms of the series, Equation (14).

This method may be used to compare the purely elastic scattering of electrons by different elements, and to determine the effect of tightly bound electrons on scattering, thus extending existing data obtained by scattering β-rays. It may be of interest to study the change in this scattering as the energy of the cathode rays is increased, so as to be able to eject more and more of the electrons in the atom. Also it might throw some light on the question of the efficiency of production of X-rays as a function of the material of the target.

II. The Polarization of the X-rays

Let $I(\phi)$ be resolved into its two components $H(\phi)$ and $V(\phi)$, polarized with the electric vectors in and perpendicular to the xy-plane respectively. Then

$$I(\phi) = H(\phi) + V(\phi)$$

and

$$H/V = I/V - 1 \ . \tag{17}$$

The intensity of the X-rays of minimum wave-length scattered at 90° to the ray perpendicular to the xy-plane can be determined experimentally. In the same way the intensity scattered at 90° to the ray but in the xy-plane may be measured. The ratio of these intensities is H/V.

We now use the second result from Bubb's theory to calculate V from the previously determined distribution of cathode rays in the target. By substituting this value of V and the experimental value of I in Equation (17) we obtain values of H/V, for all values of ϕ, which can be compared directly with the experimental values. Consequently the electric vector of any photon of minimum wave-length proceeding along OB in Fig. 1, and produced by an electron whose momentum before radiation was along OC, must lie in the plane defined by the points O, B and C. Hence the electric vector makes angle η with the xy-plane and the component of the intensity polarized with the electric vector perpendicular to the xy-plane is proportional to $\sin^2\eta$. On the directed quantum picture $\sin^2\eta$ is proportional to the probability that the photon be scattered at 90° to the ray in the xy-plane.

Then, using the notation of the previous section,

$$V(\phi) = K_1 \sin\omega\, \delta\omega \,.\, 2\int_0^{\pi} p(\psi) \sin^2\eta \, d\eta \tag{18}$$

and substituting from Equations (5), (7), (8) and (9), in Equation (18) we obtain

$$V(\phi) = -K_2 \sum_{m=0}^{\infty} A_m \frac{1}{a^2} \int_{b+a}^{b-a} \xi^m \sqrt{a^2-b^2+2b\xi-\xi^2}\, d\xi = -K_2 \sum_{m=0}^{\infty} \frac{A_m u_m}{a^2} \,, \tag{19}$$

where

$$u_m = \int_{b+a}^{b-a} \xi^m \sqrt{a^2-b^2+2b\xi-\xi^2}\, d\xi = \left[\frac{\xi^{m-1}(a^2-b^2+2b\xi-\xi^2)^{\frac{3}{2}}}{-(m+2)} + \int \xi^{m-1}\sqrt{a^2-b^2+2b\xi-\xi^2}\, d\xi \right.$$

$$\left. + \frac{(m-1)\,(a^2-b^2)}{m+2} \int \xi^{m-2}\sqrt{a^2-b^2+2b\xi-\xi^2}\, d\xi \right]_{b+a}^{b-a}$$

$$= \frac{(2m+1)b}{m+2} u_{m-1} + \frac{(m-1)\,(a^2-b^2)}{m+2} u_{m-2} \,. \tag{20}$$

Now

$$u_0 = \int_{b+a}^{b-a} \sqrt{a^2-b^2+2b\xi-\xi^2}\, d\xi = \frac{a^2}{2} \int_{b+a}^{b-a} \frac{d\xi}{\sqrt{a^2-b^2+2b\xi-\xi^2}} = \frac{a^2}{2} t_0 \,. \tag{21}$$

Also

$$u_1 = \int_{b+a}^{b-a} \xi \sqrt{a^2-b^2+2b\xi-\xi^2}\, d\xi$$

$$= b \int_{b+a}^{b-a} \sqrt{a^2-b^2+2b\xi-\xi^2}\, d\xi = bu_0 = \frac{ba^2}{2} t_0 \,. \tag{22}$$

Now Equation (19) becomes on substitution from Equation (21)

$$V = -K_2 \sum_{m=0}^{\infty} \frac{A_m}{a^2} u_m = \frac{-K_2 t_0}{2} \sum_{m=0}^{\infty} \frac{A_m u_m}{u_0}$$

or

$$V = \frac{K}{2} \sum_{m=0}^{\infty} A_m U_m ,$$

where

$$U_m = \frac{u_m}{u_0} = \frac{(2m+1)}{m+2} \cos\omega \cos\phi \; U_{m-1} + \frac{(m-1)}{m+2} (\sin^2\omega \sin^2\phi - \cos^2\omega \cos^2\phi) \; U_{m-2} .$$

$$U_0 = 1,\ U_1 = b = \cos\omega \cos\phi \text{ and, as before, } K = -K_2 t_0 . \qquad (23)$$

Here K and A_m have been determined from the intensity measurements and the values of U_m are defined. The value of V may be computed from Equation (23) and be substituted in the right-hand side of Equation (17) the check against H/V as determined by experiment.

III. The Use of a Vapor for an Effectively Thin Target

In addition to what has been mentioned, Bubb calculates from his assumptions the maximum frequency, ν_0, of X-rays which can be emitted in a direction making angle θ with the momentum vector of the cathode rays, *viz:*

$$\nu_0 = \frac{m\beta^2 c^2}{2h\sqrt{1-\beta^2}} \cdot \frac{\sin^2\theta}{1-\beta\cos\theta} \qquad (24)$$

where m is the mass of the electron. When $h\nu_0$ is equal to the total kinetic energy of an electron, $\theta = \omega$.

This relation between ν_0 and θ could be tested experimentally using a target so thin that few of the cathode rays would be scattered appreciably in passing through it. Metal foil targets are unsatisfactory for use with 30 or 40 kv. electrons, because of mechanical difficulties in their preparation, and the fact that extremely small tube currents must be used to prevent the formation of holes in them due to melting of the metal.

It was thought that the use of a mercury vapor target, whose thickness, measured in atoms, could be fixed easily at any desired value, might eliminate the difficulties. There is no limit to the tube current in this case as the target is already vaporized. An X-ray tube was built with a long, hollow anticathode cooled by water circulation. Cathode rays could pass through holes in the front and back of this anode into a chamber containing mercury, whose vapor could be kept at any desired temperature. The hollow anode served as a condenser for any mercury vapor diffusing into it, so that a high vacuum could be maintained in the fore part of the tube. The pressure of the vapor which will not cause scattering of more than any predetermined amount, can be computed or can be found experimentally, as the cathode rays excite mercury atoms, causing them to emit visible light in the beam.

The path of the cathode rays through the vapor was necessarily rather long (about 4 or 5 cm.) from the point at which the rays entered the vapor to the point from which X-rays were taken. This made necessary so low a pressure, to prevent excessive scattering, that the intensity of the X-rays was very weak. However, the X-rays could be detected quite easily photographically, but it was found impossible to keep the apparatus operating under constant conditions for a period long enough to obtain a spectrogram. It was estimated that an exposure time of 40 or 50 hr. would have been necessary to determine the minimum wave-length accurately.

A modification of the arrangement just described is contemplated in which the cathode rays will be allowed to pass through a molecular beam in which the density of atoms can be high when the cross section of the beam is small. It is hoped that the intensity of X-rays produced per unit volume (this factor governs exposure time) can be made high enough to give good spectrograms in practicable exposure times.

Acknowledgment

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REFLECTIONS FROM THE IONOSPHERE[1]

By Sydney Sillitoe[2]

Abstract

Oscillograms showing unusually low layer reflections and exceptionally strong multiple reflections from the *F* layer, and oscillograms of the usual conditions for February, March and April, are presented. Some automatic continuous records of the heights of the reflecting layers are also shown.

The main features of the new design and technique, which were important factors in the elimination of all extraneous results, are clearly pointed out.

Introduction

The original intention of the author was to develop a system of continuous recording of the apparent heights of the reflecting layers in the ionosphere with both transmitter and receiver located in the same building. As the pulse method of Breit and Tuve was to be used it was felt that attention must be paid to stability (constancy of frequency of the transmitted pulse), and that therefore a stabilized master oscillator power-amplifier circuit should be used, with only short pulses of power emitted by keying in the antenna circuit. Furthermore, for economic reasons, the whole system had to be composed of a minimum of component parts, be as simple as possible, and yet be capable of delivering sufficient power to achieve the result in mind. The last consideration was achieved. Elaborate shielding precautions had to be taken, but despite these, it was found impossible, for any reasonable cost and effort, to build filters and install shielding sufficiently effective to allow the receiver to be located in such close proximity to the transmitter.

The transmitter, therefore, remained in the attic of the Macdonald Physics Building at McGill University, and the receiving apparatus was moved about two miles away to a location in Outremont just on the other side of Mount Royal. The location of the receiver was very fortunate, as the presence of the mountain probably reduced the intensity of the ground wave, leaving the receiver more sensitive to the reflection than it otherwise would have been. Both manual and continuous recording were successfully achieved in this location, and it is the purpose of this paper to describe fully the equipment, and to show and to discuss the results obtained.

The Transmitter

Most experimenters have employed transmitter circuits in which keying was effected either in the oscillator itself or in the power supply of one of the succeeding amplifier stages. Extra vacuum tubes of equal rating had to be used as keying devices in order to eliminate arcing, and this made the installation very costly. It is a well known fact that in any oscillator, the frequency, being dependent on the internal characteristics of the vacuum tube, which in turn vary with voltage fluctuation, cannot remain constant for the full

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Contribution from the Physical Laboratories, McGill University, Montreal, Canada.
[2] *Graduate student.*

duration of a short pulse obtained by interruption of the power supply. Actually, the frequency changes as the oscillations build up and die down in the tuned circuits. This effect is still present, but less pronounced, if the keying is done in succeeding amplifier stages. But, in both cases, the wave produced is unsymmetrical, owing to the flywheel effect of the tuned circuits, which do not cease oscillating for a short time after the keying operation is over.

For these reasons stability in the transmitter was recognized as being of primary importance, and the transmitter shown in Fig. 1 was designed and built. A stabilized Colpitts' master oscillator, as suggested by F. B. Llewellyn

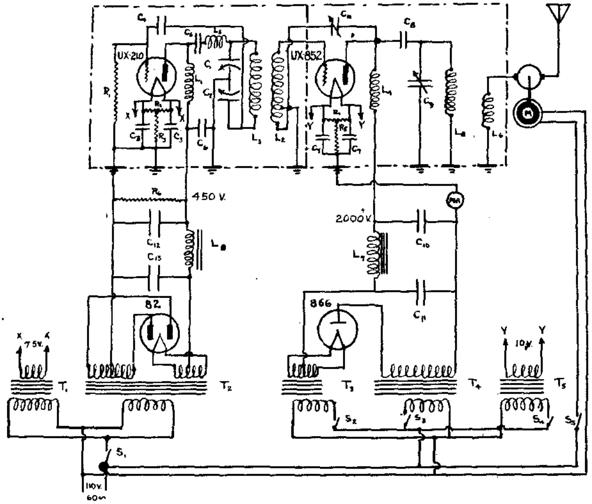


FIG. 1. *Simplified transmitter and power supply circuit.*

(7), employing a UX 210 tube to drive a UX 852—100 watt power amplifier was used. Full advantage of the oscillator stabilization was not taken, as neutralization of the UX 852 power amplifier was found necessary. Inductive coupling had therefore to be used. Self-biasing of both tubes, accomplished by resistors R_3 and R_5 in the cathode leads, was used to afford protection against overvoltage and interruption. The whole transmitter was completely shielded in a heavy copper case. By careful design a very low cost transmitter was thus produced, which operated at maximum possible efficiency and was sufficiently reliable to be left running without supervision.

Sharp, symmetrical wave pulses were radiated from the shielded transmitter by keying in the lead to the quarter-wave flat-top antenna. The commutator, driven by a synchronous motor, consisted of a fibre disk about eight inches in diameter, with three equally spaced brass segments imbedded in the surface. The width of the segments was such that it gave a pulse 0.0003 sec. long, but which could easily have been made less. Two phosphor bronze spring contacts, riding on the commutator, completed the antenna circuit for this short interval 90 times a second. This keying speed was adopted for two reasons: first, it made the framing of the picture on the oscillograph at the receiving station always possible and, second, the recurrence period of 1/90 of a second formed a convenient means of calibration, which eliminated the necessity of switching a calibration oscillator on the oscillograph in order to calculate equivalent heights. Anyone who has worked on this type of problem will appreciate the saving of time and the added convenience that this made possible. No telephone connection between stations was necessary, and it was possible for the author to operate the system alone.

In other words, keying at this speed and synchronizing the sweep with the 60 cycle power supply, made it possible to view in succession on the oscillograph screen the three transmitted pulses, corresponding to the three commutator segments. When the corresponding reflections following each transmitted pulse were identical in form and sequence, there could be no doubt that the reflections were genuine. This new technique and design were important factors in the elimination of extraneous results.

Receiving Apparatus

At the receiving station the apparatus was arranged as shown in Fig. 2. The radio receiving set used was a Silver Marshall 728 SW all wave model,

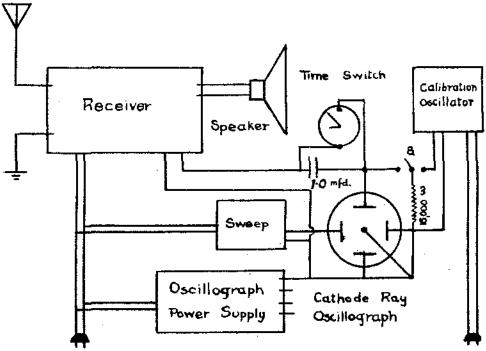


FIG. 2. *Receiver apparatus—block diagram.*

with a slight change in the output circuit. The oscillograph tube used was a Cossor type *C* fed from a General Radio cathode ray oscillograph power supply. A Cossor sweep circuit was used to supply a linear time axis.

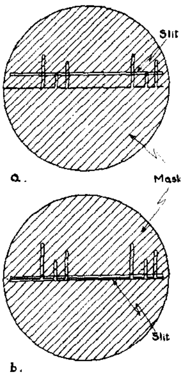


Fig. 3. *Two methods of placing the slit for continuous recording.*

The voltage developed across a 2000 ohm resistor in the plate circuit of the first audio tube of the receiver was applied through a blocking condenser (1.0 mfd.) to the ungrounded plate of the oscillograph. The ground connection to the oscillograph had to be made through the receiver which was solidly grounded. An alarm clock, with contacts placed upon the face, served as a time switch which shorted the stopping condenser and lifted up the beam for one and a half minutes at the hour points. The slit (Fig. 3), being photographed for continuous recording, was thus made blank for a long enough period each hour to make a distinct marking on the film, for the indication of the time of day at which the record was taken.

Photographs of the actual patterns on the oscillograph screen were made with a specially built box camera. Continuous records were made with a similar camera, the film being driven by a strong clockwork.

Methods of Continuous Recording

For continuous recording it is evident from Fig. 3 that there are two methods of placing the slit, depending on the type of record desired. In Fig. 3 *(a)* the slit is shown above the time line. The shutter in the camera is stopped down but left continuously open, and on the record either double or full black lines appear on a light background, depending on the intensities of the pulses. It is evident that this method records only those reflections which are above a certain intensity, and furthermore, interference conditions may completely destroy the record. When the lens is open all the time the slit cannot be placed as in Fig. 3 *(b)*, because there is a discrimination against the sustained conditions in the pattern. A transient condition, which often occurs, will bring the bright spot down to the slit and will quickly fog out a portion of the record which has almost, but not quite, completed the travel across the slit.

If the slit is placed as shown in Fig. 3 *(b)*, short exposures of one-half to one second in length must be made at intervals of one minute. This record has the advantage of being completely independent of interference conditions, as the breaks in the time line appear as white lines on a dark background, and the interference all goes into the background if the exposure is made sufficiently long. The mechanical set-up for this type of record is more elaborate. A cam arrangement is needed to actuate the shutter, and the power required for this is more than a clock can deliver.

A motor drive was used for both film and shutter, and this proved to be mechanically all that could be desired. Unfortunately the motor was the cause of a great deal of noise in the receiver and had to be discarded. A good induction motor might have served the purpose, but this was not available.

There was, however, another difficulty, in that the receiver had a recovery curve which originated in the coupling between the detector and audio stages, and this condition could not be easily eliminated. This over-swing after a strong pulse, clearly shown in Plate II, while of no particular disadvantage in a complete pattern photograph, proved nevertheless to be extremely inconvenient for continuous recording. The use of a horizontal, straight slit was impossible unless the slit was made very wide, and when this was done the record was poor. A crooked slit fitted to an average recovery curve was also useless, as this curve varied with the strength of the pulse. Other factors peculiar to the location made it impossible to obtain this type of record. Finally, it was decided to use the method shown in Fig. 3 *(a)*, as in general the reflections seemed to appear either intensely, or not at all. Moreover, the location was remarkably free from factors causing interference.

But again, when records were being made, two more difficulties arose. Changes in voltage of the power supply caused changes in the sensitivity of the oscillograph, and hence the linear amplitude of the time sweep varied; the vertical scale on the record fluctuated correspondingly. Phase changes in the power lines (which were used for synchronizing) between the transmitter and the receiver two miles away caused a creeping back and forth of the picture on the time axis—and therefore the ground level line did not appear straight as it should on the record.

Experimental Results

Most of the tests were made at a frequency of 3492 kc. A few oscillograms selected from over 500 taken are shown in Plates I and II, to represent conditions in the morning, at sunset and in the evening. Each division of the scale at the base represents 100 km. apparent height.

In Plate I the picture taken at 10:25 a.m. shows that the *E* layer and *F* layer reflections appear to come from heights of 80 km. and 207 km. respectively. The other pictures in the top row show *E* layer reflections from an average height of 105 km. In the morning, at this frequency, the *E* layer reflections were neither continuous nor strong, and, as shown, at 11:01 a.m. and 11:41 a.m., had died out completely. Strong, continuous *F* layer reflections from heights ranging from 207 to 233 km. appear, followed by weaker multiple reflections. Some synchronous interference causes the pulse located at 800 km. on the scale.

In the afternoon these same conditions prevail, but the *E* layer generally completely disappears except for very short intervals now and again.

Shortly after 5:10 p.m. in the months of February and March, a most interesting period for observation was almost always found. ***Many transient conditions appear just before the sunset hour.*** Suddenly, strong reflections

appear from the F layer, and the pattern on the screen of the oscillograph rolls like the waves on the ocean as the reflections and their many multiples appear and disappear. For short periods now and then, the E layer also reflects. Such conditions are clearly shown at the bottom of Plate I and the top of Plate II. Pictures such as those taken on March 15 are quite common and show the strong F layer reflection coming from an average height of 250 km. Many multiple reflections appear and disappear.

The two pictures taken on March 14 at 5:50$_{40}$ p.m. and 5:51 p.m. show an exceptional transient condition which was luckily photographed. These pictures, taken in rapid succession, show first the F layer and six multiple reflections, with F_5 and F_6 alternately dying out. Suddenly at 5:51 an intense E layer reflection appeared, which apparently was again reflected from the earth, to pierce the E layer and be subsequently reflected from the F layer producing all the multiple $E + F$ reflections shown. The duration of this condition was never more than half a minute. Note the intensity of the reflected sky waves in comparison with that of the ground wave. These are not the only photographs obtained of such conditions, but have been selected from a few pictures showing the same unusual condition.

In Plate II progressive stages of magneto-ionic splitting from the F region are shown for March 23. *These reflections are of a very transient nature and are rising and falling continually,* first on one side, and then on the other. Three and four pulses come in close together, and it is not strange that short wave messages are not clear under such conditions. At 9:45 p.m. on March 19, a very stormy night, the E (?) layer reflection was exceptionally strong and gave a multiple reflection also. Note that it is exceptionally low, 50 km. The condition shown existed, from time to time, for short intervals over a period lasting about three hours. Low layer reflections were also photographed on three other days, but the intensities were much less than in the example shown in Plate II.

In Fig. 4 the results obtained throughout the day in February and March have been plotted. The upper and lower boundaries of the regions from which reflections were obtained are shown. The F layer is closest to earth at noon, being as low as 160 km. The E layer reflections were relatively weak at this frequency (3492 kc). but the height of the layer is generally about 100 km. However, it should be noted that, at odd times in the afternoon and evening, reflections were returned from heights as low as 40 km.

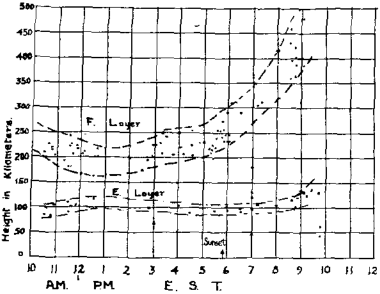
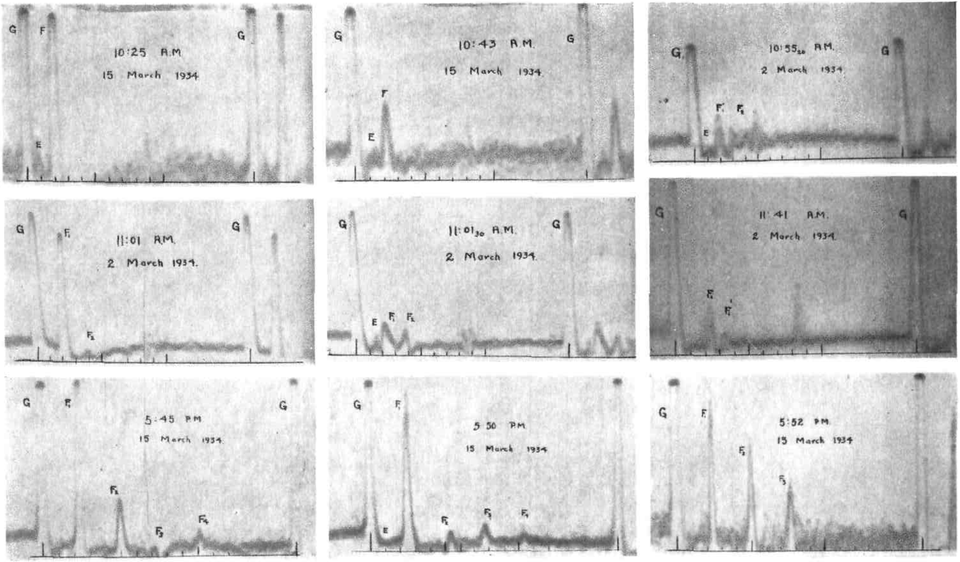


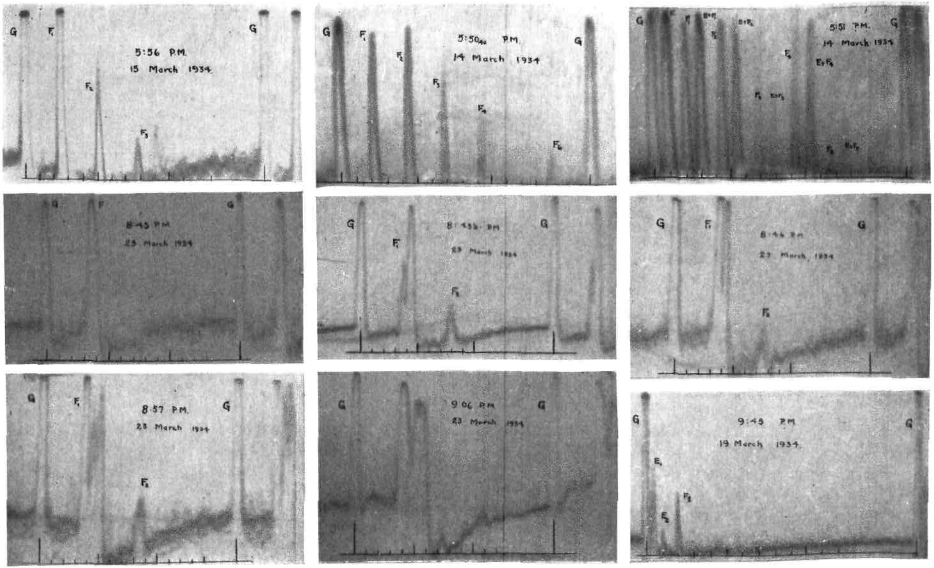
FIG. 4. *Variation in the heights of the reflecting regions with time of day. February and March, 1934. Frequency—3492 kc.*

PLATE I

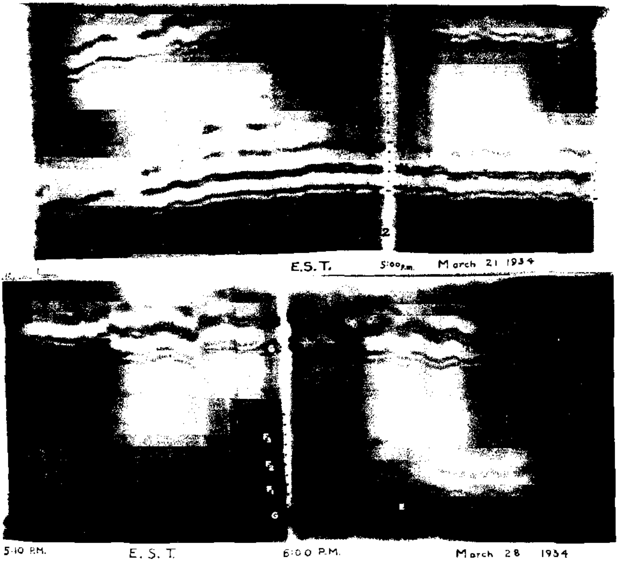


Oscillograms. Frequency 3492 kc.

PLATE II



Oscillograms. Frequency—3492 kc.



Continuous recordings of heights of reflecting layers. Frequency—3492 kc.

The results of a test run at a frequency of 2398 kc. are plotted in Fig. 5. In the morning the reflections were very weak, but at times the *E* layer seemed to be very low. The *E* layer appeared to rise from 100 km. at noon to 140 km. at 5 p.m. In the evening scattered reflections were obtained from as low as 70 km., and from as high as 160 km. The reflections from the *F* layer were very weak in the morning, but when they were present, the height was between 200 and 250 km. In the evening the reflections became much stronger and the height gradually increased. At 9:30 p.m., the interference became too great for successful photography.

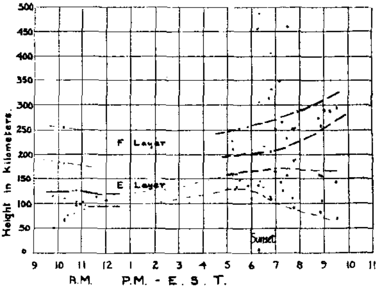


FIG. 5. *Variation in the heights of the reflecting regions with time of day. April 2 and 3, 1934. Frequency—2398 kc.*

In Plate III a section of a continuous record obtained on March 28 is reproduced. These records show directly the same type of information indicated in Figs. 4 and 5. The equivalent heights in 100 km. are shown on the hour lines. The difficulties encountered in obtaining this type of record, as already pointed out, are clearly apparent. Voltage and phase changes in the 60 cycle power lines were responsible for the fluctuations in the position and recurrence of the ground wave. Although, on this account it is in a somewhat inconvenient form, the information is nevertheless always available on these records. They do not give the same amount of information as the complete oscillogram, and at a frequency of 3492 kc. only unusually strong *E* reflections could be recorded. The recovery curve of the receiver also discriminated against the recording of the lower *E* layer echoes. When splitting occurred the echo lines were broadened out and less distinct.

Conclusions

In general, the results of these tests agree with those obtained by previous experimenters. However, on account of the short pulse and stability of the transmitter, it was possible to record reflections from the *E* (?) layer when apparently it was as low as 40 km. above the ground. In February, March and April, these reflections from an exceptionally low layer were observed on only four days. These were not continuous, but appeared from time to time, especially after sunset, and possibly in the early morning up to 10 a.m., and were generally weak when observed. The strength of the reflections and the unusual conditions shown in Plate II are quite interesting, and *indicate the quick changes that must take place in the ionosphere during certain periods.* The presence of a sixth multiple *F* reflection of good intensity shows the

efficiency of the reflecting or refracting layer and the relatively small absorption. It is curious to note that this wave actually travelled about 3000 km., but has progressed only two miles from the transmitter in so doing.

It is also a curious fact that an apparent multiple reflection may die out, while the two adjacent echoes remain. This effect was generally noticed near sunset and sometimes suggested, for short periods, another layer extremely high up, about 1100 km. The explanation of the fact that F_4, for example, has not returned to earth, while F_5 has successfully done so, may be that the reflection of F_4 took place from the top of a low E layer, which was sometimes observed, as already pointed out in the discussion of Fig. 4. Indeed, at other times, echoes were observed coming apparently from heights corresponding to $E + F$.

The results of tests at different frequencies indicate that the higher frequencies are more penetrating. In a short run at 4360 kc., made April 4, in the late afternoon, echoes from the F layer at a height of 360 km. were obtained, while at 3492 and 2398 kc. at corresponding times of day, the heights were found to be generally about 250 and 235 km. respectively. This is in accordance with the continuous observations over a wide frequency range made by Kirby, Berkner and Stuart.

Acknowledgments

The author wishes to express his sincere appreciation of the hospitality accorded him by Mr. and Mrs. J. T. Aikman and family, who so kindly gave space in their home for the location of the receiving equipment. He is greatly indebted to Mr. H. T. Pye and Mr. G. Tweeddale who assisted in building parts of the transmitter and camera, and to Mr. S. Amesse who photographed the plates shown in the paper. The helpful discussions with Dr. H. G. I. Watson and Dr. A. S. Eve were of definite value.

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TETRAPHENYLCYCLOPENTADIENONE IN THE DIENE SYNTHESIS[1]

By C. F. H. Allen[2] and L. J. Sheps[3]

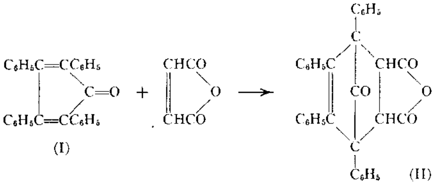
Abstract

In continuation of the authors' earlier work on substances having a carbonyl bridge, it has been found that tetraphenylcyclopentadienone can be used as one of the components in the diene synthesis. Products are described resulting from the addition of maleic anhydride, maleic, fumaric, and acetylene dicarboxylic esters, and benzoyl phenylacetylene. In some instances the primary addition products containing a carbonyl bridge could be isolated. Tetraphenylbenzene derivatives result from secondary reactions. Benzalacetophenone, dibenzoylethylene and diphenylketene did not add to tetraphenylcyclopentadienone.

In the previously described reaction between maleic anhydride and anhydroacetonebenzil, certain intermediates have been isolated, confirming the mechanism suggested to account for the formation of a dianhydride.

It was recently shown in this laboratory that when cyclopentadienones dimerize the product is formed by a diene syntheses and has a carbonyl bridge across a six-membered ring (3, 4). On account of the ease of dimerization, a monomolecular form has never been isolated except in the case of tetraphenylcyclopentadienone (6, 14). In accordance with the assumption made in the previous paper, the latter would be expected to enter into the diene syntheses with some of the usual addends, and, as previously announced (3), it was found that it does.

When tetraphenylcyclopentadienone, I, and maleic anhydride are cautiously warmed together, an addition product, II, is formed.



When the reaction mixture is heated to a somewhat higher temperature, the bridge is lost as carbon monoxide, and a new anhydride, III, obtained. Finally, when the mixture is heated to 250° C. during the preparation, both carbon monoxide and hydrogen are lost, and tetraphenylphthalic acid, IV, is

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Contribution from the Chemical Laboratory, McGill University, Montreal, Canada.
This paper was read at the meeting of the Royal Society of Canada at Quebec, May, 1934, and at the Toronto meeting of the Canadian Chemical Association, June, 1934.

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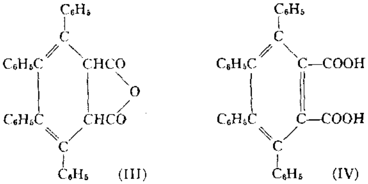
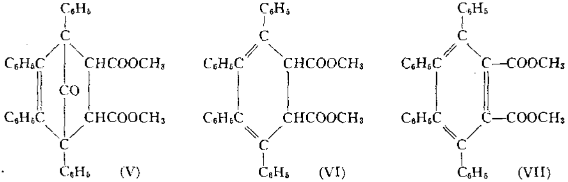
the substance actually isolated; this same product is also obtainable by heating II and III, with or without sulphur. Therefore, three series of products may be obtained, according to experimental conditions.



(III) (IV)

The anhydrides and acid can be hydrolyzed and esterified; the esterification takes place slowly as with any hindered acid. When the anhydride II was treated with alcoholic hydrogen chloride it gave a mixture of mono and dimethyl esters; the diester was more conveniently prepared through the silver salt.



(V) (VI) (VII)

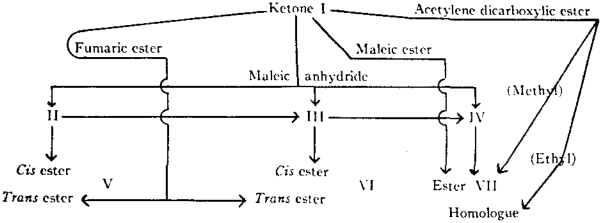
The ester, V, being derived from maleic acid, would be expected to have the *cis* configuration; that it actually has is conveniently shown by the preparation of the *trans* ester, by the addition of methyl fumarate to tetraphenylcyclopentadienone. Ester VI is also *cis;* the *trans* isomer was obtained by conducting the addition of methyl fumarate at a high enough temperature to eliminate the bridge as carbon monoxide.

When methyl maleate is used as an addend, however, it is not possible to stop the reaction at any intermediate point, both carbon monoxide and hydrogen are lost, and the aromatic ester, VII, is formed. Whether the difference is due to the higher temperature of reaction required, or to some other reason, is uncertain.

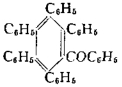
Finally, to show that two atoms of hydrogen had been actually lost, and that VII is a benzene derivative, it was synthesized from tetraphenylcyclopentadienone and acetylene dicarboxylic (methyl) ester; the product was identical with the substance prepared in other ways. The ethyl ester was also prepared; in this case heating in a sealed tube was required. Owing to the higher temperature required to bring about the addition of these esters, the primary product containing a carbonyl bridge could not be isolated. All

efforts to secure the ethyl ester by esterification of the acid were unsuccessful, nor could it be hydrolyzed to this acid. It is a very stable substance, being recovered unchanged after heating with caustic potash at 360° C.

The relation between the various substances is much clearer, when summarized in a flowsheet.

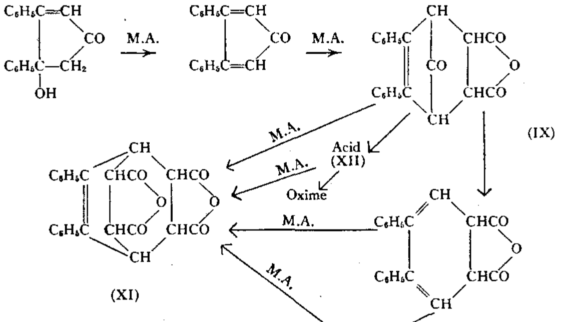


Neither benzalacetophenone nor dibenzoylethylene adds to tetraphenylcyclopentadienone, but benzoylphenylacetylene gives pentaphenylbenzophenone, VIII. As would be anticipated, this ketone has a very inactive carbonyl group.



(VIII)

In the diphenylcyclopentadienone series, a dianhydride, XI, has previously been described (4); this may be accounted for as shown by the outline



M.A.= Maleic anhydride.

After the authors' preliminary notice on the di- and tetraphenyl series, and a paper on the diphenylcyclopentadienone series, had appeared, Dilthey (6, 8) published two short papers, almost devoid of experimental details, in which were described certain highly arylated benzene derivatives prepared from tetraphenylcyclopentadienone and several acetylenic compounds, and three anhydrides in the maleic anhydride series. It is of interest to note that in no single case, either in his or the authors' work, has a substance having a carbonyl bridge been obtained with acetylenic addends.

The intermediate anhydrides are oils, but the corresponding acids are solids. When the acid XII is heated, it loses carbon monoxide; the product XIII contains a conjugated linkage, to which maleic anhydride easily adds to form XI; the latter also results on heating IX and maleic anhydride. This behavior of these compounds is comparable to that of coumalin and maleic anhydride, in which case carbon dioxide was lost (5). Acid XII also forms an oxime, thus confirming the carbonyl group.

Staudinger found that ketenes and cyclopentadiene form addition products that might be represented as cyclobutane derivatives, or as endomethylene compounds, if a diene synthesis had taken place (10, pp. 23, 59; 11, 12, 13). If diphenylketene added to tetraphenylcyclopentadienone, this would offer evidence in favor of the latter possibility. However, the present authors have been unable to find any experimental conditions under which addition would take place, which, indirectly, favors the cyclobutane formulas. Phenyl azide, a reagent used to detect "active" ethylenic linkages (1, 2), does not add to any of the substances containing a carbonyl bridge in either series, possibly on account of the hindrance of the phenyl groups.

Experimental

A. Addition of Maleic Anhydride

(a) Anhydride (II) of 3,4,5,6-tetraphenyl-7-keto-1,2,3,6-tetrahydro-3,6-methanobenzene-1,2-dicarboxylic acid

A mixture of 5 gm. each of maleic anhydride and tetraphenylcyclopentadienone (hereafter referred to as the red ketone) was cautiously heated in an oil bath at 155–160° C., taking care not to exceed this temperature, until the red color had practically disappeared (five minutes). After cooling, the mass was readily removed if it was first loosened by addition of methanol and then gently warmed. After recrystallizing from acetic acid or benzene, 6 gm. (96%) of a pink product was obtained. The color disappeared after repeated treatments with a decolorizing carbon. It formed white plates, m.p. 220° C.* It is insoluble in the ethers and alcohols, moderately soluble in hot dioxane, acetic acid, benzene, and ethyl acetate, and very soluble in warm acetone and cyclohexanone. Calcd. for $C_{33}H_{22}O_4$: C, 82.1; H, 4.5%; mol. wt., 482. Found: C, 81.7; H, 4.7%; mol. wt., 486.

It was not possible to obtain an oxime or a phenylhydrazone. The results obtained with the Grignard machine were anomalous; until the behavior of other anhydrides is thoroughly investigated, their interpretation is impossible. Tetraphenylcyclopentadienone itself shows one addition, and tertiary alcohols have been prepared from it using phenyl and benzylmagnesium halides (9, 14).

The corresponding *cis* acid dissolves readily in the alcohols, ether, benzene, acetic acid, and decalin; the amorphous product melted at 190–191° C., with evolution of gas. Calcd. for $C_{33}H_{24}O_6$: mol. wt., 500. Found: mol. wt., 511. The silver salt shrivels and blackens at 185–190° C. Calcd. for $C_{33}H_{22}O_6Ag_2$: Ag, 30.2%. Found: Ag, 30.1%.

* *Melting points are corrected.*

The *cis* dimethyl ester, V, was obtained by refluxing 1 gm. of the silver salt with 5 cc. of methyl iodide for 0.5 hr., filtering and evaporating, and recrystallizing from dilute methanol, after treating with animal charcoal; yield, 0.65 gm. It forms a micro-crystalline powder which shrinks at 135° C., and gives off a gas at 155° C. Calcd. for $C_{35}H_{28}O_5$: OCH_3, 11.7%. Found: OCH_3, 11.3%.

The anhydride was dissolved in absolute methanol and saturated with hydrogen chloride for five hours at 0° C. After 24 hr. a solid, which melted at 120–122° C. with gas evolution, was obtained. Methoxyl values (8.4%) were intermediate between those of a half ester (6.0%) and the whole ester (11.7%,) but the solid could not be separated into its components. When 0.5 gm. was heated at 145–155° C. for a few minutes and worked up by appropriate manipulation, 0.3 gm. of the anhydride II resulted; the latter was presumably formed by loss of alcohol from the *cis* half ester. On the assumption that the conversion was quantitative, the calculated methoxyl value for the mixture is 8.2%.

(b) Anhydride (III) of 3,4,5,6-tetraphenyl-1,2-dihydro-o-phthalic acid

The anhydride II (1 gm.) was heated for 10 min. in an oil bath at 235–240° C., and the product crystallized from a chloroform-methanol mixture. It formed shining hexagonal plates, m.p. 255° C.; the yield was 1.4 gm. (82%). When acetic acid or benzene was used as solvent, a semi-gelatinous form was obtained. Calcd. for $C_{32}H_{22}O_3$: C, 84.6; H, 4.8%. Found: C, 84.2; H, 5.1%.

cis Methyl 3,4,5,6-tetraphenyl-1,2-dihydrobenzene-o-phthalate, VI. Dry hydrogen chloride was passed through a solution of 0.4 gm. of the anhydride in 40 cc. of absolute methanol at 0° C. for two hours. The solution was then refluxed for one hour. The next day the solvent was partially removed, and 0.3 gm. (68%) of the ester separated from the cold solution. It separated in shiny triangular plates from methanol, m.p. 149–150° C. Calcd. for $C_{34}H_{28}O_4$: OCH_3, 12.4%. Found: OCH_3, 12.2%.

(c) 3,4,5,6-Tetraphenyl-o-phthalic acid, IV

A mixture as in (*a*) was heated at 240–250° C. as long as gas was evolved, and treated with methanol, followed by recrystallization from acetic acid. Yellow rods were obtained, from which the color was removed with difficulty by repeated treatment with decolorizing carbon; m.p. 287° C. Calcd. for $C_{32}H_{22}O_4$: C, 81.7; H, 4.7%; mol. wt., 470. Found: C, 81.3; H, 4.6%; mol. wt., 468, 475. The acid is insoluble in the alcohols, and ether, but dissolves readily in hot ethyl acetate or benzene.

This acid also resulted in yields of 60–78% by heating either of the other anhydrides, II or III, alone or with sulphur at 260–270° C.

**Dilthey (8) gives the melting point 286° C., but analytical figures for the corresponding anhydride.*

The sodium salt separated as silky needles on cooling a hot solution of the acid in a 20% sodium hydroxide solution containing a little alcohol. The acid was regenerated by acidifying the hot solution.

Methyl 3,4,5,6-tetraphenyl-o-phthalate, VIII. Dry hydrogen chloride was passed through a suspension of 1 gm. of the acid in 40 cc. of dry methanol at 0° C. for three hours. After a week the ester had been formed in a yield of 90%; it crystallized in rosettes, m.p. 250–251° C. Calcd. for $C_{34}H_{26}O_4$: OCH_3, 12.4%. Found: OCH_3, 12.1, 12.2%.

It was impossible to secure the ethyl ester by esterification of the acid by any of the usual procedures, but it was obtained as described below.

B. Addition of Methyl Maleate

This same ester, VII, was obtained in a yield of 61% when the red ketone was heated with twice its weight of methyl maleate at 165° C.

C. Addition of Methyl Fumarate

(a) Trans methyl ester, V

Equal weights (2 gm. each) of the red ketone and methyl fumarate were well mixed and heated gently up to 170° C. by means of an oil bath. On cooling, the solid was boiled with 25 cc. of methanol and filtered hot. The residue was taken up in 35 cc. of hot dioxane, and an equal volume of methanol was added. On standing, 1.9 gm. (63%) of fine prisms separated; m.p., 214–215° C. with evolution of gas. Calcd. for $C_{35}H_{28}O_5$: C, 79.5; H, 5.3%. Found: C, 79.5; H, 5.0%.

(b) Trans methyl 3,4,5,6-tetraphenyl-1,2-dihydrobenzene-o-phthalate, VI

By heating the above mixture at 225° C. as long as gas was evolved, and recrystallizing from methanol, a 70% yield of this ester was obtained; it formed needles, m.p. 157° C. Calcd. for $C_{34}H_{28}O_4$: C, 81·6; H, 5.6%. Found: C, 81.3; H, 5.6%. It was also obtained on heating the ester,V; there was some reversal of the addition reaction, since some of the red ketone was also formed.

D. Addition of the Acetylene Dicarboxylic Esters

The methyl ester, VII, was prepared by cautiously heating a mixture of the red ketone and methyl acetylene dicarboxylate over a free flame. The mass was boiled with methanol, evaporated to dryness, and the gummy product washed free from oil with ether; the yield was 31%.

Ethyl 3,4,5,6-tetraphenyl-*o*-phthalate was formed in a 22% yield when 2 gm. of the red ketone and 4 gm. of the ethyl ester* were heated in a sealed tube for two hours at 300–310° C. The resulting mixture was boiled with alcohol, which left the unused ketone as a residue, decolorized with animal charcoal, and crystallized from a dioxane-alcohol mixture. It separated in

* *The acetylene dicarboxylic ethyl ester was kindly supplied by the National Aniline and Chemical Co.: their assistance is gratefully acknowledged.*

clusters of stubby rods, m.p. 187–188° C. Calcd. for $C_{36}H_{30}O_4$: C, 82.1; H, 5.7%. Found: C, 82.1; H, 5.4%. These components did not react at the boiling point of the ester.

This ester is very stable. It was recovered unchanged after being heated to 360° C. with molten potassium hydroxide, as well as after 27 hr. of refluxing with a 40% solution of aqueous alcoholic potash.

E. Addition of Benzoyl Phenylacetylene

2,3,4,5,6-Pentaphenylbenzophenone, VIII

A mixture of equal weights (0.5 gm. each) of benzoyl phenylacetylene and the red ketone was heated slowly to 195° C., in a closed system to measure the gas evolved, and kept at this point for five minutes; 29.8 cc. of gas was collected (calcd. 28.8 cc.). The pink reaction mixture was washed with methanol, leaving a 93% yield of a white product that, on recrystallizing from a dioxane-methanol mixture, formed diamond-shaped prisms, m.p. 340–341° C. It dissolved readily in the usual solvents except methanol. Calcd. for $C_{43}H_{30}O$; C, 91.8; H, 5.3%. Found: C, 91.4; H, 5.4%. It did not form an oxime, and it does not show any addition in the Grignard machine.

F. Miscellaneous

It was found impossible to add benzalacetophenone and *trans* dibenzoylethylene to the red ketone at the boiling point of the mixture, the components being recovered unchanged.

Diphenylketene

The operations were all conducted in an inert atmosphere. In no case was there an apparent reaction, the red ketone being entirely recovered. The conditions were varied widely, but prolonged contact at either low or high (250° C.) temperatures did not lead to the desired result.

G. Anhydracetonebenzil and Maleic Anhydride*

4,5-Diphenyl-7-keto-1,2,3,6-tetrahydro-3,6-methanobenzene-1,2-dicarboxylic Acid, XII

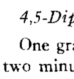
A mixture of 10 gm. each of anhydracetonebenzil and maleic anhyride was heated three to five hours in an ethylene bromide bath (b.p. 131° C.), and, while still hot, dissolved in 225–300 cc. of hot 7% potassium hydroxide solution. The solution was then allowed to cool and filtered. On addition of 80 cc. of concentrated hydrochloric acid an oil separated. The oil was taken up in ether and shaken with sodium carbonate solution. On acidifying the latter with concentrated hydrochloric acid a viscous oil was precipitated; after standing overnight the aqueous layer was decanted. The oil was taken up in alcohol, the solvent evaporated and the amorphous residue dissolved

* *Some of the preliminary experiments were performed by Dr. E. W. Spanagel.*

in chloroform; Skelly Solve C* was added until the solution became cloudy, and the whole allowed to evaporate spontaneously. The viscous mass was dissolved in hot benzene, cooled, and poured into Skelly Solve C with rapid stirring. The precipitate was collected and the process repeated 6–12 times, until an almost white amorphous product was obtained. It was then recrystallized from dilute alcohol until the melting point was 186–187° C. with gas evolution. The yield was 61–65%. The product formed shiny flakes, very soluble in the alcohols and ether, but less soluble in benzene and chloroform. When 10% hydrochloric acid was used in the acidification, the acid potassium salt separated. The silver salt, prepared in the usual manner, melted at 198–199° C. with decomposition. Calcd. for $C_{21}H_{16}O_5$: C, 72.4; H, 4.6%; mol. wt., 348; potassium salt, 9.2% potassium; silver salt, 38.4% silver. Found: C, 72.8, 72.6; H, 4.5, 4.8%; mol. wt., 347; K, 9.4; Ag, 38.5%.

The oxime was obtained by boiling for 27 hr. a mixture of 1 gm. of the acid and the filtered solution resulting from a mixture of 2 gm. of hydroxylamine hydrochloride, 3 gm. of potassium acetate, and 20 cc. of hot alcohol. After standing overnight, 0.65 gm. of prisms separated and was recrystallized from alcohol; m.p. 219–220° C. with decomposition. Calcd. for $C_{21}H_{17}O_5N$: N, 3.8%;—for $C_{21}H_{15}O_4N$: N, 4.1%. Found: N, 4.0%.

Attempted esterification by several methods, iucluding the use of diazomethane, yielded oils that could not be purified. When a solution in absolute methanol was saturated with hydrogen chloride, a solid, m.p. 145° C. after recrystallization from methanol or benzene, was isolated. It was insoluble in sodium hydroxide solution and was recovered unchanged after shaking with concentrated ammonium hydroxide. Its structure was not determined; the analytical values are not far from those of a ketal-diester, XIV.



(XIV)

Calcd. for $C_{25}H_{26}O_6$: C, 71.1; H, 6.2; OCH_3, 29.4%. Found: C, 70.1, 70.5, 70.0; H, 5.3, 4.9, 5.2; OCH_3, 28.7, 30.0%.

4,5-Diphenyl-1,2-dihydro-o-phthalic acid, XIII

One gram of the acid XII was heated in an oil bath at 190° C. for one to two minutes, the glassy product taken up in hot benzene, and poured into an equal volume of Skelly Solve C with rapid stirring. The amorphous product was filtered and recrystallized from *p*-cymene. This gave a very fine powder, m.p. 235–243° C. with decomposition; yield, 1.1 gm. (65%). It dissolves readily in the usual organic solvents except petroleum ether. The silver salt had a melting point of 205° C. with decomposition. Calcd. for $C_{20}H_{16}O_4$: C, 75.0; H, 5.0%; silver in silver salt, 40.4%. Found: C, 74.7; H, 4.9; Ag, 40.2%. This acid also gave intractable oils on esterification.

Both acids, when heated with maleic anhydride at 200° C., gave the dianhydride XI. The behavior of these dianhydrides on heating, alone or admixed with an authentic sample, and that of the pure dianhydride, were the same.

* *Petroleum ether fraction, b.p. 90–100° C.: the Skelly Oil Co.*

H. Phenyl Azide

Mixtures of phenyl azide, a solvent, and all of the compounds with a carbonyl bridge, described in this and the previous paper, were kept in sealed tubes at room temperature for three to four months. In every instance, the substances were recovered unchanged.

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STUDIES ON HOMOGENEOUS FIRST ORDER GAS REACTIONS

V. THE DECOMPOSITION OF PARACETALDEHYDE AT HIGH PRESSURES[1]

By A. L. Geddes[2] and C. C. Coffin[3]

Abstract

The homogeneous first order gaseous decomposition of paraldehyde to acetaldehyde has been studied at temperatures from 230 to 254° C. up to pressures at which the liquid phase makes its appearance, *i.e.*, 12 atm. at 230° and 18 atm. at 254° C. Over-all velocity constants for the homogeneous reaction in the heterogeneous liquid-vapor system have been determined from these pressures up to the critical point. The data confirm results already published. It is found that in the purely gaseous system increase of pressure tends to diminish the reaction velocity. That the specific reaction velocity in the liquid phase is greater than that in the vapor phase is shown by the fact that the velocity constants of the heterogeneous systems increase progressively with the liquid-vapor ratio. Extrapolation to 100% of liquid gives velocity constants about five times as great as those characteristic of the vapor phase. Peculiarities in the behavior of the system at the critical point and preliminary measurements of the velocity of the trimolecular reverse reaction are described.

Introduction

Measurements of the rate of decomposition of paraldehyde to acetaldehyde at pressures up to, and beyond, the critical were undertaken for several reasons. In the first place it is of considerable importance to find a third order gas reaction suitable for study, and in this respect the reverse of the paraldehyde decomposition looked promising (1). It is also of interest to follow the rate and mechanism of such a simple clean-cut reaction through as large a concentration range as possible, as it is not clear from existing data to what pressures the limiting "high pressure rate" of a first order reaction may be extrapolated. The scarcity of data with regard to the rates of a first order reaction in the gaseous and liquid states, particularly in view of the catalytic effect associated in some types of reaction with the presence of the liquid phase (4, 9), was a further incentive to the undertaking of this work. Moreover, it was considered desirable to check the data already obtained for this decomposition (1) by an experimental procedure as different as possible from that previously employed. Such a check is particularly necessary in the case of a reaction as susceptible to traces of impurities as the paraldehyde decomposition appears to be.

It must be stated at once that the above objects are still far from being completely attained. The present paper is concerned only with reaction velocities in the vapor state up to pressures where the liquid phase appears, and with a preliminary exploration of the heterogeneous liquid-vapor system up to, and beyond, the critical point.

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Contribution from the Laboratory of Physical Chemistry, Dalhousie University, Halifax, Nova Scotia, Canada.

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Experimental

Purification of the Paraldehyde

Paraldehyde from three different sources was purified in several different ways. As no consistent difference in reaction velocity was evident among these samples, details of the methods employed need not be given. It is sufficient to state that the essential step in each case was one of the following:—recrystallization, fractional distillation at atmospheric pressue and *in vacuo*, treatment with metallic sodium and distilling, or freezing several times in a high vacuum and subsequently subliming at low pressures.

Rate Measurements at the Lower Pressures

Velocity constants were calculated from the changes in composition that occurred when bulbs containing paraldehyde were kept for a definite time in a thermostat. In the case of runs below about 20 atm. the reaction bulbs were made from wide (2–3 cm.) Pyrex tubing and were shaped as shown at *B* in Fig. 1. The thermostat, *H*, was an air bath consisting of a Pyrex Dewar flask (25 by 10 cm.) heavily lagged with shredded asbestos and connected to an electrically heated mercury boiler and a water cooled reflux condenser as shown in the diagram. The flask was lined with heavy sheet copper to increase its heat capacity and to protect it in case of an explosion. The pressure on the boiling mercury and in the 20-litre stabilizing volume (connected at *S*) was read on the manometer *M* and could be adjusted by manipulation of taps connected to a source of dry air or to an evacuating system. Temperatures were determined from the vapor pressure curve of mercury.*

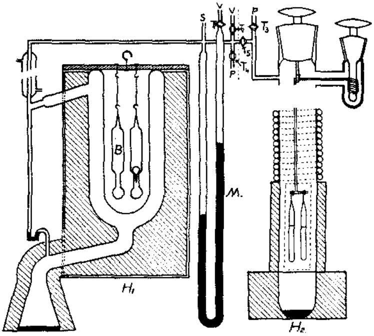

FIG. 1. *Diagram of apparatus.*

In the experiments at the lower pressures a sealed Pyrex capsule containing a weighed amount of paraldehyde was introduced into the open end of the reaction tube which was then drawn down, sealed to a manifold, thoroughly evacuated and sealed off. The capsule burst and the reactant vaporized when the bulb was lowered into the thermostat.

*This form of oven has been found to be very convenient for obtaining constant temperatures between 200 and 300° C. It has many obvious advantages over a liquid bath especially in this temperature range. The disadvantage that reaction bulbs do not reach the thermostat temperature as rapidly as when plunged into a liquid was eliminated here by working at temperatures low enough for the uncertainty about zero time to be negligible in comparison with the total time.

Great pains were taken in cleaning and evacuating the reaction bulbs. In most cases the bulbs were cleaned with hot chromic acid mixture, thoroughly washed with distilled water and flamed *in vacuo* to a temperature near their softening point. In some instances all washing was avoided, the glass being simply wiped out with cotton plugs and flamed as before. As the treatment to which the bulbs were subjected, or the addition of broken glass, had no appreciable influence on the rate of decomposition, it is concluded that the reaction is homogeneous.

In the experiments at pressures greater than 10 atm. the empty reaction bulbs were sealed to a manifold and evacuated. They were then connected with a reservoir of carefully purified paraldehyde at room temperature and were kept at $-78°$ C. until a suitable quantity had condensed in each bulb, which, after melting and freezing its contents several times *in vacuo*, was sealed off. The amount of paraldehyde in each bulb was determined at the end of the experiment by weighing the bulb before and after opening. Volumes were determined after opening by filling with water. Pressures were calculated from the gas laws.

After a definite time in the thermostat the reaction tubes were removed and the small bulbs at the bottom (see *B*, Fig. 1) were immersed as quickly as possible in a carbon dioxide-ether mixture to freeze out the contents. The bulbs were hung by hooks (shaped when sealing off after filling) from the heavy asbestos thermostat cover which could be raised or lowered from a distance by means of a system of strings and pulleys. The operator was thus out of range of the explosions which sometimes occurred as a result of the system cooling too rapidly. The bulbs were usually left in the thermostat for a length of time sufficient to allow the reaction to progress approximately to half-value. The extent to which the paraldehyde had decomposed was determined by one or both of two different methods.

1. The small bulb at the end of the reaction tube was cut off and dropped, while its contents was still frozen, into a stoppered flask containing 50 cc. of ice-cold sodium bisulphite solution of known iodine titre. The solution was titrated with standard iodine after it had stood for half an hour at 0° C. Preliminary experiments had shown that the method gave consistent and reliable results provided that the equilibrium between sodium bisulphite and acetaldehyde (8) was taken into account.

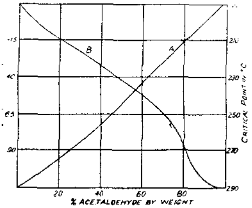

FIG. 2. *Curve A,—Critical temperatures of paraldehyde-acetaldehyde mixtures. Curve B,—Melting points of paraldehyde-acetaldehyde mixtures.*

2. The melting point of the bulb contents was determined and the composition of the mixture was interpolated from Hollmann's data (6) on the freezing points of paraldehyde–acetaldehyde mixtures (Curve B, Fig. 2). An alcohol-ether mixture vigorously stirred by means of a current of dry air and contained in a large transparent Dewar flask served as the melting point bath. The temperature

was regulated by the addition of "dry ice" or alcohol, and was measured on an alcohol thermometer graduated in $\frac{1}{5}$° C. and calibrated for total immersion over its whole range against a standardized thermocouple. Only the small bulbs at the bottom of the reaction tubes were immersed—the remainder of the tube being at room temperature. As the melting point was approached the temperature was allowed to rise very slowly and the contents of the bulbs was kept well mixed by vigorous shaking. The error due to vaporization of acetaldehyde was shown to be negligible for all temperatures and bulb volumes employed.

The extent to which the two analytical methods agree is shown in Table I, in which the velocity constants of some typical experiments at 230° C. are listed. As explained below the pressures given in Column 1 are the initial pressures that would have prevailed had all the paraldehyde vaporized. Actually, above 12 *atm.* liquid was present at this temperature during part of the time the bulbs were in the thermostat and the calculated pressure is simply taken as a measure of the over-all concentration. Columns 2 and 3 give the velocity constants determined by the titration method for reaction periods of three and six hours respectively. Constants as determined from the melting point after three and six hours in the thermostat are listed in Columns 4 and 5. In cases where both methods were used for the same bulb the melting point was of course determined before titrating.

TABLE I

TYPICAL FIRST ORDER VELOCITY CONSTANTS DETERMINED BY DIFFERENT METHODS AND FOR DIFFERENT TIMES OF REACTION. TEMPERATURE 230° C.

Initial press., atm.	$k \times 10^5$ by titration method		$k \times 10^5$ by melting point method		$k \times 10^5$ average	Initial press., atm.	$k \times 10^5$ by titration method		$k \times 10^5$ by melting point method		$k \times 10^5$ average
	Time, 3 hr.	Time, 6 hr.	Time, 3 hr.	Time, 6 hr.			Time, 3 hr.	Time, 6 hr.	Time, 3 hr.	Time, 6 hr.	
1.6	—	4.9	5.2	4.3	4.8	11.5	—	—	4.1	4.1	4.1
1.9	—	4.3	4.7	3.8	4.2	11.4	—	—	3.8	4.0	3.9
2.8	5.8	—	5.6	—	5.7	13.1	—	—	4.1	4.2	4.1
3.8	5.0	—	4.9	—	4.9	14.9	5.4	—	6.0	—	5.7
3.3	5.5	—	5.3	—	5.4	22.1	—	5.5	6.1	5.3	5.6
7.4	3.9	—	3.6	—	3.8	26.3	7.3	—	—	—	7.3
7.8	3.9	—	3.8	—	3.8	23.8	—	—	7.1	—	7.1
8.2	3.7	—	3.5	—	3.6	25.7	—	—	6.7	—	6.7
9.9	3.5	—	3.4	—	3.4	25.9	—	—	7.5	—	7.5
6.6	4.2	—	4.2	—	4.2	34.9	—	—	8.5	—	8.5
10.4	4.4	—	4.1	—	4.3	30.0	—	—	8.5	—	8.5
10.9	—	3.5	3.5	3.3	3.4	27.8	—	—	7.7	—	7.7
11.6	—	4.4	4.1	4.1	4.2						

It will be observed that for any one bulb the two methods agree as well as can be expected in work of this kind. In the later experiments the melting point method only was employed. After the experiments, in all cases, the contents of the bulbs were kept frozen until they could be analyzed.

The analytical methods described above were used in the case of bulbs in which the calculated initial pressures were less than about 15 atm.

If the volume of liquid in the bulb were less than a certain percentage of the total volume, the liquid disappeared on attaining the temperature of the thermostat and the reaction proceeded in the vapor phase. If the volume of the liquid were above this percentage, the liquid phase persisted until enough paraldehyde had decomposed to allow complete vaporization. These limiting volumes were determined by filling tubes to various levels and observing the time taken for the liquid to evaporate at the temperatures in question. The curves obtained were extrapolated to zero time and the corresponding initial percentage volumes were read from the graph. The results are shown in Table II. At these temperatures therefore bulbs containing volumes of liquid greater than these limiting percentages yielded "composite" velocity "constants"—part of the constant representing the velocity in the liquid phase, and part that in the vapor phase. These constants will be discussed later.

TABLE II

Temp., °C.	Initial % volume of liquid (at room temp.)	Calculated pressure of paraldehyde at temperature of thermostat, atm.
230	4	12.5
242	5	15.7
254	6	19.0

It was observed also that if a bulb more than 15% full of liquid at room temperature were placed in the thermostat, the critical composition of the system was reached before all the liquid had evaporated, *i.e.*, the liquid phase finally disappeared not by evaporation but by disappearance of the meniscus. If a bulb were more than about 50% full at room temperature it became filled with liquid before the critical composition was reached, and of course burst. In the case of bulbs between 15 and 50% full, therefore, over-all velocity constants were obtained by the following method which is based on the fact that the meniscus becomes invisible at any one temperature when a definite composition is reached.

Rate Measurements at the Higher Pressures

The bulbs were made of 2–3 mm. bore heavy walled Pyrex capillary tubing and were filled as before by distillation *in vacuo*. The reaction was carried out by immersing the bulbs directly in mercury vapor in the thermostat H_2 (Fig. 1), which consisted of a three-foot length of two-inch Pyrex tubing connected as shown to manometer, evacuating system, etc. The bulbs could be lowered quickly into, or removed from, the mercury vapor (below the copper cooling coil) by means of a hoist, the operation of which is obvious from the diagram. The entire length (10–15 cm.) of the bulbs was visible through the double window (dotted rectangle) cut in the asbestos insulation. During the reaction they were observed through a cathetometer behind a heavy plate glass shield. The time taken for the meniscus to disappear was noted and the composition of the mixture when this occurred was inter-

polated from Hollmann's (6) critical temperature curve (curve *A*, Fig. 2) for the two-component system. The total volume of the bulb and the initial volume of liquid paraldehyde at room temperature were estimated to a sufficient degree of accuracy with the cathetometer. First order velocity constants for the reaction in the heterogeneous system were calculated from the time taken to reach the critical composition.

These experiments exhibited some interesting features. During the reaction the position of the meniscus changed as shown in Fig. 3. The numbers on the curves of Fig. 3, *A*, (242° C.), and 3, *B*, (254° C.), are the percentages of the total bulb volume occupied by the liquid at room temperature. The ordinates give the change (in centimetres) in the height of the meniscus during the reaction and are calculated to a bulb length of 10 cm.; the zero is the position occupied by the meniscus at the temperature of the thermostat before any reaction has occurred. The abscissas represent the time; the end of each curve is the point at which the meniscus became invisible. All observations were made with a cathetometer.

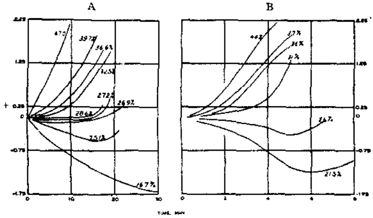

FIG. 3. *Change of position of meniscus with time at 242° C. (A) and at 254° C. (B).*

It will be observed that when a bulb more than about 27% full of liquid at room temperature is placed in the thermostat the meniscus steadily rises until it flattens out, becomes wider and less distinct, and finally disappears. If a bulb is less than 27% full the meniscus falls as the reaction proceeds until just before its disappearance, when it begins to rise. This rise does not occur until the surface has become mobile and hazy and is unmistakably a relatively broad density gradient. The same upward drift of the meniscus just before the critical point is reached occurs also in bulbs slightly more than 27% full and is the only movement of the meniscus during the reaction in bulbs just 27% full. It is noteworthy that Maass and coworkers (10) have observed that bulbs about 27% full of methyl ether, ethylene and propylene can be raised to the critical temperature without change in the position of the meniscus. This point is being considered more fully in another paper.

The appearance of the critical phenomenon differed somewhat from that usually observed in the case of one-component systems. In all bulbs, streams of bubbles much too small to appreciably affect the stability of the meniscus ascended continually throughout the reaction and became invisible when the meniscus did; no opalescence was visible as the critical composition was reached and passed. If, however, the bulbs were allowed to cool slowly and uniformly after the liquid phase had vanished, the phenomena associated with the reappearance of the meniscus were practically identical (except for

the bubbles) with those observed by Travers and Usher (11), for example, in their study of one-component systems. Thus if the meniscus had vanished near the top of the bulb the fog appeared in the space that had been occupied by the vapor; when it had disappeared near the bottom of the bulb the fog became visible in the space that had been occupied by the liquid. In the case of tubes 27% full the fog was uniformly distributed throughout the whole volume. Bulbs cooled so that a temperature gradient existed along their length frequently presented a very curious appearance for a few moments; *e.g.*, two and even three liquid surfaces were often present at the same time.

It should be pointed out that a polymerization reaction occurs to an appreciable extent if the liquid phase remains for a sufficient length of time. Thus at 230° C. the critical phenomena cannot be observed as the high boiling polymer has time to be formed in quantities sufficient to prevent the change of state. The polymer is dark red in color and according to Hollmann (6) its presence is readily detected by the yellow tinge it imparts to the liquid.

The Reverse Reaction

The critical point method described above is being used also to measure the velocity of the reverse reaction, *viz.*, the condensation of gaseous acetaldehyde to paraldehyde. Here the situation is more favorable for such a method, in that the system remains homogeneous until the composition is indicated by the appearance of the liquid phase and the experiment is completed. Preliminary experiments have given third order constants between 2 and 3 $\times$ 10^{-6} at 210.6° C. (nitrobenzene vapor).

Discussion of Results

The velocity constants obtained for the paraldehyde decomposition are plotted as ordinates in Figs. 4 (230° C.) and 5 (242 and 254° C.). The abscissas in Fig. 4 are the initial pressures that would have existed had all the paraldehyde vaporized. As already stated, when the calculated pressure is above about 12 atm. at 230° C. the liquid phase persists for a length of time which is proportional to the amount of paraldehyde present. A velocity constant above this calculated pressure therefore does not represent a true velocity in any one phase, but it may be regarded as a measure of the rate at which the heterogeneous system as a whole tends to reach equilibrium. The fact that these "constants" increase with the amount of liquid phase present indicates that the specific reaction velocity is greater in the liquid than in the vapor phase. It is to be noted that the minimum in the curve of Fig. 4 corresponds approximately to the point at which the systems become heterogeneous.

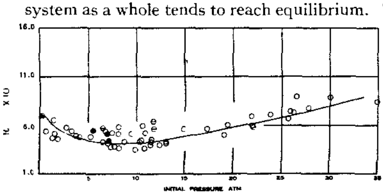

FIG. 4. *Variation of velocity constant with pressure at 230° C.*

In Fig. 5 the velocity constants at 242 and 254° C. are plotted against the initial percentage volume of paraldehyde in the bulbs at room temperature. The data below 15% volume were obtained by either melting points or titrations as already described. The contents of the bulbs more than 15% full were caused to react in the thermostat H_2, and were "analyzed" by observing the time taken for the meniscus to disappear. In these curves also the reaction velocity begins to increase after the liquid phase makes its appearance.

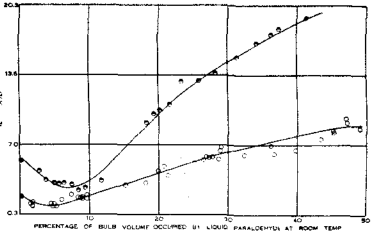

FIG. 5. *Velocity constants plotted against initial percentage volume of liquid. Upper curve,—254° C. Lower curve,—242° C.*

From the good agreement shown in Table I between the velocity constants determined for the same bulb after three hours' (approximately 50% reaction) and after six hours' (approximately 75% reaction) at 230° C., it is apparent that the decomposition remains first order for any one experiment over the pressure range in question. Table III which shows the velocity constants for different times of reaction at 242° C. is further evidence that this is the case. The time to half-value at 242° C. is roughly one hour.

TABLE III

FIRST ORDER CONSTANTS FOR DIFFERENT REACTION TIMES AT 242° C. COMPOSITIONS WERE CALCULATED FROM MELTING POINTS

% volume of paraldehyde at start (room temperature)	$k \times 10^4$ after 1 hr.	$k \times 10^4$ after 1.5 hr.	$k \times 10^4$ after 2 hr.	Mean $k \times 10^4$
1.6	1.4	1.3	—	1.4
1.8	1.2	1.0	—	1.1
1.9	1.5	1.3	—	1.4
8.3	1.9	—	1.7	1.8
8.7	2.0	—	1.7	1.8
9.0	1.9	—	1.6	1.7
9.4	2.2	—	1.9	2.0
9.7	2.4	—	2.3	2.3
9.7	1.8	—	1.7	1.7

The points ⊙ on the ordinates of Figs. 4 and 5 are the velocity constants calculated from the previously published (1) rate equation, *viz.*,

$$\ln k = 34.83 - \frac{44200}{RT}.$$

The good agreement between the results obtained by such widely different experimental methods is evidence that a true uncatalyzed reaction velocity is being measured. The points ● (Fig. 4) were obtained with bulbs containing a drop of mercury; the bulbs with which the points ⊖ were obtained were filled with finely broken glass. It is thus apparent that mercury vapor and glass surface are without appreciable effect on the rate of decomposition. Runs at 230° C. between 5 and 30 cm. pressure were made in an apparatus described elsewhere (2), with each of the six different preparations of paraldehyde used in obtaining the data of Fig. 4. As these experiments all gave constants between 6.5 and 6.9 $\times$ 10^{-5} it is probable that no catalytic impurity was present in any of the samples of paraldehyde used, and that the wide scattering of the points in Fig. 4 is due to errors in the rather difficult analyses.

The most striking feature common to the curves of Figs. 4 and 5 is the apparent drop in the velocity constant at any one temperature as the pressure is increased up to the point where the liquid appears. This decrease in velocity is so small that it is observable only over a large pressure range. It is however believed to be real and is being more carefully investigated. The scarcity of data with regard to clean-cut first order reactions at high pressures makes such a study particularly desirable. The authors have already suggested (3) that in the case of complex molecules, in which the energy distributions most probable on collision are not coincident with those most favorable to reaction, an increase in the number of collisions may mean a decrease in the rate of reaction. Kassel, however, has shown (7) that such a mechanism is unlikely. Experiments designed to reveal the cause of this effect and to discover to what extent it is characteristic of the decomposition of complex molecules are in progress.

From the curves of Figs. 4 and 5 it is evident that the specific reaction velocity is greater in the liquid than in the vapor phase. A rough idea of the velocity in the liquid phase may be obtained by extrapolating the curves for the heterogeneous systems to 100% liquid volume (66–69% volume at 20° C.). The results of the extrapolation are given in Table IV. The velocity constants in the vapor phase at pressures below one atmosphere (column 3) are calculated from the rate equation (1).

TABLE IV

FIRST ORDER VELOCITY CONSTANTS IN LIQUID AND VAPOR PHASES

Temp., ° C.	k (liquid phase)	k (vapor phase)
230	3×10^{-4}	6×10^{-5}
242	1×10^{-3}	2×10^{-4}
254	2.5×10^{-3}	5.5×10^{-4}

It will be observed that the specific reaction rates in the liquid state are about five times as large as those in the vapor state at the three different temperatures. It would thus appear that the temperature coefficients and hence the activation energies are the same in each phase, and that whatever may be the mechanism underlying the catalytic activity of the liquid state it is

not to be ascribed to a lowering of the activation energy. It must be remembered, however, that the uncertainties in the extrapolated values are so large that the figures of Table IV can be of significance only in so far as order of magnitude is concerned. Moreover, as a fall in E of 2000 calories would be more than sufficient to increase the reaction velocity by a factor of five, the necessity of more complete data in this connection is obvious.

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STUDIES ON THE OVERWINTERING OF CERTAIN FUNGI PARASITIC AND SAPROPHYTIC ON FRUIT TREES[1]

By L. W. Koch[2]

Abstract

Evidence is submitted that some conidia of the black knot fungus, *Dibotryon morbosum* (Sch.) T. and S., are capable of overwintering in a viable condition on *Prunus domestica*. *D. morbosum* has also been isolated on numerous occasions during the winter from definite chlamydospores on buds and bark of *P. domestica*. Pure cultures of *D. morbosum* produced chlamydospores only when grown in close proximity to certain other organisms, or when certain chemicals were introduced into the Petri dishes. Chlamydospore formation was also induced on twigs of *P. domestica* when conidia of *D. morbosum* germinated in intimate association with a strain of bacteria originally isolated from the same host. *Cladosporium carpophilum* was isolated in several instances from chlamydospores on the bark of *Prunus persica*. This organism also produced chlamydospores both on culture media and on peach twigs. A histological study revealed the presence of two types of twig lesion. Early infections produced primary lesions during the summer, and secondary lesions during the following winter as a result of the migration of the mycelium beyond the barriers of periderm delimiting the primary lesions. Late infections, on the other hand, rarely induced periderm formation and did not produce secondary lesions during the winter. *Taphrina deformans* was isolated during the winter from the surface of buds on *P. persica*. Proof is submitted that spores of *T. deformans* can remain viable throughout the winter on twigs of the above-mentioned host. Other fungi, presumably saprophytes, representing the genera *Coniothyrium, Cladosporium, Hormodendrum, Fumago* and a fungus impossible to identify because it produced chlamydospores only, all developed from chlamydospores occurring on various hosts. The latter fungi all produced chlamydospores in culture. The evidence obtained indicates the probability that many more fungi overwinter as chlamydospores than have been reported to do so. Certain misapplications of the term chlamydospore in the various groups of fungi are dealt with. Owing to their unsatisfactory mycological status the importance of chlamydospores has hitherto been incompletely appreciated. The results of these investigations emphasize the importance of the application of a dormant spray on fruit trees.

Introduction

The mode of overwintering of fungi parasitic on fruit trees is an unusually interesting problem, especially in view of the fact that certain of them have not been shown to produce a perfect stage. In the case of those organisms which do produce a perfect stage during the winter the ascospores are generally accepted as the only viable winter spore form and the possibility of the overwintering of other spore forms remote in time and place from the parent mycelium has not been investigated.

The present investigation was undertaken primarily to discover whether any of the fungi causing diseases of fruit trees overwinter by any method or methods other than those already reported. These investigations, conducted over the four-year period 1930-1934, evolved from a study of black knot, the causal organism of which was demonstrated in 1930 to be capable of overwintering in a form hitherto unreported. The studies were then extended to include not only *Dibotryon morbosum* (Sch.) T. and S., but also *Cladosporium*

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carpophilum Thüm., and *Taphrina deformans* (Fcl.) Tul., as well as certain saprophytic members of the Fungi Imperfecti. The latter were included because their method of overwintering was found to be similar to that of the pathogenes. For the purpose of clarity each organism has, for the most part, been dealt with separately in the present article.

Materials and Methods

Since in the investigations outlined below both the choice of materials and technique employed were the same for the different organisms concerned, a general outline of the methods and materials involved will suffice.

For purposes of isolation, buds and bark from branches of all ages were removed from *Prunus domestica*, *P. cerasus*, *P. americana*, *P. persica*, *Pyrus communis* and *Pyrus malus*. Earlier in the investigations various methods for freeing the fungous populations from the snrfaces of buds and bark were attempted. These consisted of soaking in water overnight, centrifuging, etc., but the method finally adopted as most successful involved the moistening of the surfaces of buds or bark with sterile water for a period varying from 15 to 30 min. The moistened surface was then scraped lightly with a sterile scalpel and the cuticular and epidermal tissues thus removed were suspended in a drop of sterile water on a sterile slide and macerated thoroughly. The latter process was most necessary for the separation of the various organisms that were present. Loopfuls of the suspension were streaked on the surface of potato-dextrose agar in Petri dishes, the medium having been centrifuged prior to pouring to remove the larger opaque particles. By using dilutions of appropriate concentrations it was usually not difficult to remove separately the fungi which developed on the plates. Careful exploratory work with the microscope at frequent intervals during the subsequent few days revealed the presence of fungi in the form of (i) fragments of mycelium, and (ii) dark, thick-walled spores probably in the resting condition. Certain of these germinated quickly, others more slowly. This necessitated their removal at successive intervals to prevent one from overrunning the other. All were removed by the method employed by Keitt (9).

Dibotryon morbosum (Sch.) Sacc.

Since 1821 when Schweinitz (16) described the perithecial stage of the organism causing black knot, the fungus, since named *D. morbosum*, has been assumed to overwinter only in the form of mycelium perennial in those infected branches of *Prunus* spp. on which perithecia develop in the winter. The only other method which to date has received consideration by other investigators has been the possible overwintering of conidia produced the previous summer. The consensus of opinion in this regard, as expressed by Selby (17), has been that the conidia are incapable of remaining viable through the winter.

In the present study the possibility of the overwintering of conidia has been reinvestigated and other means have also been sought by which the fungus might perpetuate itself throughout the dormant season.

Conidia

To test the viability of conidia after exposure to winter conditions, tube cultures of the conidial stage as well as masses of dry conidia of *D. morbosum* in Petri dishes were placed outdoors about October 1 for three successive years. At the same time, suspensions of conidia were painted on buds and on surface-sterilized twigs, particularly at nodes. In these experiments only the most easily recognized strain of *D. morbosum* was used. Re-isolations from all of the above-mentioned materials were made at intervals of three weeks throughout the winter.

Particular care was taken to identify the pathogene on the twigs because another closely related fungus was frequently isolated from apparently normal twigs of *P. domestica*.

Periodic platings of materials lightly scraped both from the roughened surface of knots and from that of the bark adjacent to knots, consistently yielded isolants of *D. morbosum*, thus lending confirmatory evidence as to the ability of some conidia, at least, to remain viable throughout the entire winter.

In parallel experiments it was found also that conidia of *D. morbosum* placed in dry, sterile Petri dishes remained viable at room temperatures for 388 days, and in a refrigerator at $-6°$ C., for at least 420 days.

To investigate the possibility of *D. morbosum* overwintering on buds or bark of *Prunus* spp. series of isolations were made at intervals during four consecutive winters. In the case of *P. domestica* isolations were made from check trees in the laboratory orchard (11) which were severely infected with black knot. Isolations from *P. cerasus* were made from buds and bark of specimens obtained from severely infected trees in unsprayed orchards in the Georgian Bay district. The results of these isolations are summarized in Table I.

TABLE I

SUMMARY OF RESULTS OF ISOLATIONS OF *D. morbosum* FROM *P. domestica* AND *P. cerasus*

Time of isolation	Source of isolations	Frequency of occurrence of organism	
		From monochlamydospores*	From undetermined sources
October to April	Scrapings from bark of *P. domestica*	42	115
October to April	Scrapings from buds of *P. domestica*	20	60
October, December and April	Scrapings from bark 6–10 in. from knots on *P. cerasus*	12	23

**The term "chlamydospore" as used in this article in connection with the fungi under investigation may be defined as a single or many-celled, thick-walled body, frequently with dense contents, sometimes highly vacuolated, borne on non-specialized, vegetative mycelium either in a terminal or intercalary position, and germinating by a germ tube.*

It will be observed in Table I that *D. morbosum* appeared 272 times in isolations made during four successive winters from the surface of buds and bark of *P. domestica* and *P. cerasus.* In 74 cases this organism developed from single chlamydospores and while the origin of the other 198 was not definitely determined, undoubtedly many of these also were of similar origin.

It is interesting to note in the above experiments: (i) that a fungus, identified as the conidial stage of *D. morbosum*, remained viable throughout the winter on buds and bark, and (ii) that in many instances the organism was perpetuated by chlamydospores, a spore form hitherto unreported for this fungus.

It should be pointed out here that in connection with all isolations involving chlamydospores, extreme care was taken to select single chlamydospores. In some instances, however, it was impossible to distinguish single compound chlamydospores from small groups possibly consisting of single-celled chlamydospores. In the case of intercalary chlamydospores difficulty was also experienced in separating the latter from mycelial fragments.

Artificially Induced Production of Chlamydospores by D. morbosum

In the face of the fact that *D. morbosum* developed from chlamydospores on buds and bark of *P. domestica*, although chlamydospores have never been reported in connection with the life history of this fungus, it was essential to demonstrate the capability of the organism to produce chlamydospores in culture.

The subjection of pure cultures of the organism growing on different media to a wide range of environmental conditions, particularly of temperature and moisture, failed to induce chlamydospore production. It was finally discovered, however, that an abundance of chlamydospores invariably developed in cultures to which certain organisms or chemicals were introduced.

(a) Production of Chlamydospores in the Presence of Certain Organisms

Petri dishes containing potato-dextrose agar were streaked on one half with conidia of *D. morbosum* and on the opposite half at some distance away, either (i) with suspensions of certain bacteria isolated from bark of *P. domestica* or (ii) with conidia of *Cephalothecium roseum* isolated from the surface of black knots (11). Both organisms grew normally for a few days, after which time, however, the growth of the mycelium of *D. morbosum* nearest to the bacteria or to *C. roseum* was arrested. This phenomenon was observed even when the organisms were separated by the distance of one inch, when there was less than 10 cc. of medium in the Petri dish in which the organisms were growing. Shortly after the arrest of growth an abundance of chlamydospores both intercalary and terminal (Plate I, Figs. 1–7) appeared on the mycelium of *D. morbosum* in closest proximity to the antagonistic bacteria, or to *C. roseum.*

A series of experiments in which suspensions of conidia of *D. morbosum* and of the antagonistic bacteria were intimately mixed and streaked on the surface of potato-dextrose agar resulted in similar but more marked results.

In these cases the conidia germinated slowly, but instead of normal germination, the majority formed typical, large, chlamydospore-like bodies either in direct association with the conidia or at the distal end of a short germ tube (Plate II, Figs. 6, 8, 9). These bodies were thick-walled and highly vacuolated, and became gradually darker in color with age. At intervals up to three weeks they were transferred to fresh acidified agar where a proportion of them always germinated and developed normal colonies of *D. morbosum*.

In another series of experiments small squares of agar were removed from just beyond the periphery of (i) colonies of the antagonistic bacteria and (ii) colonies of *D. morbosum*. The former were then transferred to the spaces previously occupied by the latter. It was observed that the cubes of agar removed from the proximity of the bacteria exerted the same inhibiting influence and induced chlamydospore formation as effectively as the bacteria themselves. Filtrates from the bacterial cultures also produced a similar though less marked effect.

(b) Production of Chlamydospores by the Introduction of Chemicals into Petri Dish Cultures of D. morbosum

Certain chemicals including sulphuric, nitric and hydrochloric acids, copper sulphate crystals, potassium and sodium nitrate and paradichlorobenzol were introduced into Petri dishes containing colonies of *D. morbosum*. Two of these chemicals, namely, sulphuric acid and copper sulphate, more particularly the former, induced chlamydospore formation.

When one drop of 75% sulphuric acid was introduced at the edge of a Petri dish in the centre of which was growing a young colony of *D. morbosum* the growth of the hyphae on the side of the colony nearest the acid was arrested. Twelve hours afterwards the beginning of chlamydospore formation was observed. The growth of the hyphae on the opposite side of the colony, on the other hand, appeared to be quite normal. Chlamydospore formation in these cultures, in some cases after a week or more, was followed by the formation of sclerotium-like bodies (Plate II, Fig. 10). In some instances where sulphuric acid was introduced into plates containing large amounts of media (about 15 cc.) extraordinarily large chlamydospore-like bodies developed. These bodies, much larger than either those observed outdoors or those more commonly observed in cultures, were almost spherical in shape, dark brown and usually thick-walled and vacuolated (Plate II, Figs. 1 and 4), and germinated by germ tubes in the same manner as those previously described (Plate II, Figs. 2, 3 and 5).

(c) Outdoor Experiments

To determine whether or not chlamydospore formation in *D. morbosum* could be induced outdoors on branches of *P. domestica*, water suspensions of conidia of the former mixed with antagonistic bacteria were painted on surface-sterilized, current-season twigs in September and October. As controls, other twigs, some of which were surface-sterilized and others not, were painted with pure suspensions of conidia of *D. morbosum*. The spore-

covered surfaces of some of each set of twigs were wrapped, first with moist blotting paper and then with wax paper, to ensure moist conditions for germination while others were allowed to remain under natural conditions.

On all twigs painted with the mixtures of conidia and bacteria the germinating conidia developed chlamydospores similar to those observed in cultures. On the exposed twigs chlamydospores developed later and were fewer in numbers. On twigs painted with conidia only, chlamydospores were observed in small numbers on non-sterilized twigs but to a much lesser extent on sterilized ones.

Discussion

In summarizing the results of the experiments outlined above, conclusive evidence is adduced that some conidia of *D. morbosum* remain viable all winter on the buds and bark of *Prunus* spp.; also that *D. morbosum* can and does overwinter in some instances in a spore form hitherto unreported, namely, the chlamydospore. Not only was this organism isolated on numerous occasions from a random selection of single chlamydospores removed from the surface of buds and bark of *Prunus domestica* and *P. cerasus*, but it was also induced to form chlamydospores by (i) association with certain other organisms, and (ii) the introduction of certain chemicals.

The fact that either filtrates of antagonistic bacteria or portions of medium from just beyond the periphery of the bacterial colonies themselves induced a similar, though less marked effect, suggests the formation of a metabolic product by the bacteria, capable of stimulating the production of chlamydospores in *D. morbosum*.

When sown in direct contact with antagonistic bacteria, both in culture and outdoors on the surface of twigs and buds, conidia of *D. morbosum* in many cases produced chlamydospores immediately after germinating. On the other hand, pure suspensions of conidia of *D. morbosum*, treated in a manner similar to that described above for the mixed organisms, resulted in the formation of no chlamydospores in culture and only small numbers outdoors.

The evidence pointed out in the above paragraphs suggests the strong possibility that when a conidium of *D. morbosum* germinates on the surface of a twig in close proximity to an antagonistic organism the former soon develops a chlamydospore. It is also quite conceivable that upon the removal of the antagonistic organism the chlamydospore may germinate in the normal way provided other conditions are favorable.

Cladosporium carpophilum Thüm

Keitt (10), who has made important contributions to our knowledge of peach scab, observed that the causal organism, *C. carpophilum*, overwinters for the most part in the form of mycelium in lesions on living twigs. He suggests that mycelium of the fungus may also survive winter conditions saprophytically, for he states "the mycelium of this fungus may quite probably survive the winter under orchard conditions upon fallen fruits, twigs or leaves".

With these possibilities in mind an investigation of the overwintering of *C. carpophilum* was undertaken. Sections of twig and leaf pustules were examined critically at frequent intervals during all seasons, isolations were made from various host organs and the organism was studied in culture.

Isolations of C. carpophilum from the Surface of Peach Twigs

During the winter of 1931 numerous isolations were made from the surface of twigs of *Prunus persica*, variety Late Crawford. The twigs were chosen from two trees which had been very severely infected with scab and which, except for a single application of spray in 1933 to control leaf curl, had not been sprayed during the three-year period of the investigations. In the first series of isolations, four isolants of *C. carpophilum* developed in plates inoculated with material scraped from the surface of twigs. During the following two years, 16 isolants of the scab organism were obtained in a similar manner and seven of these were definitely determined as originating from single chlamydospores. In the latter instances, germination of the chlamydospores was so tardy and the growth of the resultant colonies so slow that their identity could not be established until 18 to 24 days after they were planted on the medium.

In December 1932, groups of chlamydospores and conidia of *C. carpophilum* were observed in macerations of material scraped from the axillary region of buds on twigs heavily infected with scab. Iudividual chlamydospores and conidia were isolated from suspensions of the macerated material, streaked on plates of solid medium and transferred to tubes. Eight isolants of *C. carpophilum* appeared in culture, six of them developing from chlamydospores, the other two from conidia. A large number of both types of spores did not germinate.

Production of Chlamydospores by C. carpophilum

(a) In cultures.—On potato-dextrose agar (dextrose, 3%) it was observed that *C. carpophilum* produced an abundance of chlamydospores, while on sterilized peach twigs microsclerotia were produced in equal abundance, in accordance with the observations of Keitt (10).

(b) On twigs.—To determine whether or not *C. carpophilum* could be induced to form chlamydospores on its host, heavy suspensions of conidia were painted in September of two successive years, on the surface of peach twigs, some of which had been surface-sterilized and others of which had been left untreated. Some of the spore-painted surfaces were wrapped with moist blotting paper enclosed by an outer wrapping of wax paper, while others were left exposed. Examinations of these twigs ten days to one month later showed that of the conidia which had germinated, some had produced chlamydospores soon afterwards. From the latter *C. carpophilum* was re-isolated in a few cases.

Pathological Histology of Lesions on Twigs and Leaves

To study the relation of *C. carpophilum* to its host, numerous sections of both twig and leaf lesions were made at close intervals throughout their developmental period. Fresh materials were utilized in all cases. Sections of twigs were cut with a sliding microtome while those of leaves were cut by the freehand method, and all were stained with cotton blue.

Examination of sections of infected twig material showed that: (i) in earlier stages of infection a mycelial mat of varying thickness occupied a position between the cuticle and the epidermis over the whole area of the lesion, with occasional hyphal strands ramifying between the epidermal cells (Fig. 1, *A*); (ii) in later stages of early current-season infection, the production of an abundance of conidia resulted in the rupture of the cuticle, and mycelium was present in abundance only at the periphery of the lesions where it still occupied the same relative position between the cuticle and the epidermis; (iii) except in very young lesions the superficial diseased tissue was completely delimited from the underlying, healthy cortical tissue by a periderm of varying thickness, which developed just in advance of the mycelium of the fungus.

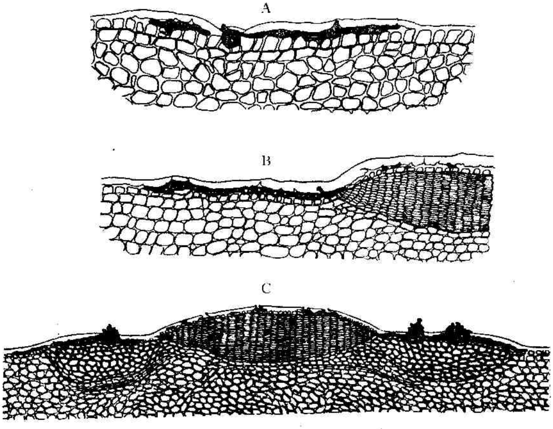

FIG. 1.

Diagrammatic representations of longitudinal sections of lesions caused by Cladosporium carpophilum on twigs of Prunus persica.

A. *Early stage of infection. Note position between cuticle and epidermis, occupied by mycelium.* × *45. Section cut 14.7.32.* B. *Later stage of infection. Note that mycelium has migrated beyond primary lesion delimited by periderm.* × *45. Section cut 17.11.33.* C. *Lesion approximately one year old showing primary and secondary lesions. Note position occupied by mycelium, and beginning of periderm formation beneath both primary and secondary lesions.* × *25. Section cut 20.6.33.*

In late season infections the lesion does not progress much beyond the initial stage, rarely induces periderm formation, and produces few or no conidia during the first season.

Examination of a large number of sections indicated that until the end of the growing season the fungus does not appear beyond the limits of the periderm induced by the action of the organism. Early in the dormant season, however, tissue development in the host ceases altogether or proceeds very slowly, while on the other hand, the fungus continues to advance between the cuticle and the epidermis, into which region periderm formation does not extend. Consequently by October the mycelium of the fungus has usually passed beyond the primary lesion, which may be identified by the underlying periderm (Fig. 1, B). During the winter and the following spring, the fungus advances slowly and produces in the newly invaded tissues what may be termed a secondary lesion (Fig. 1, C). The latter can be distinguished macroscopically as an irregular ring of tissue which is of a darker red color than the surrounding tissues and which gradually becomes elevated as the winter progresses. The walls of the host cells beneath the mycelium in the young secondary lesion become thickened but it is not until the following spring that a true periderm is laid down, delimiting the secondary lesion which is now clearly distinguishable from the primary lesion. During the second summer the fungus fruits most abundantly and in most cases only within the limits of the secondary lesions. Early in the second growing season the tissues underlying the primary lesions resulting from late summer infection closely resemble those below the secondary lesions described above. At the same time conidia are produced over the whole surface of the infected area, in marked contrast to the early season infections.

Examinations of sections of leaves showed a condition approximating that on twigs. During the summer definite lesions result from infection. The mycelium of *C. carpophilum* occupies a subcuticular position and is frequently delimited from the surrounding healthy tissues in much the same manner as on twigs. By fall, however, the mycelium has frequently advanced beyond the boundaries of the primary lesion where in many cases it fruits abundantly.

Discussion

These observations supply evidence that *Cladosporium carpophilum* may overwinter on the surface of peach twigs in the form of chlamydospores in addition to the well recognized and undoubtedly more important method of overwintering as mycelium in lesions on twigs.

When it is considered that *C. carpophilum* produces an abundance of chlamydospores in culture it is not surprising that the same spore form has been found outdoors; nor is it surprising that the chlamydospores of this fungus are capable of overwintering, since it has been observed by Keitt (10) that chlamydospores of this organism are highly resistant to desiccation. He cites one instance of chlamydospores in a gradually dried culture remaining viable for more than three years.

A critical examination of lesions on twigs has revealed certain interesting information. Twig lesions can be roughly divided into two classes: (i) early infections which produce conidia during the season of infection, and (ii) later infections which produce few or no conidia until the second season. Lesions of the first type produce secondary lesions during the winter as a result of the advance of the fungous mycelium beyond the primary lesion. During the whole course of infection the organism occupies a position between the cuticle and the epidermis.

Taphrina deformans (Fcl.) Tul.

Among numerous other fungi appearing in isolations from buds and bark of *Prunus persica*, an organism resembling *Taphrina deformans* appeared on five occasions, occurring first during the winter of 1930. As the actual method by which *T. deformans* overwinters was in doubt and as this organism is somewhat difficult to distinguish in culture from certain "budding" saprophytic fungi, many of which appear consistently in isolations from the bark of peach trees, it was necessary to prove the identity of the organism by inoculation. For this purpose two trees were chosen, both of which had been sprayed in the fall of 1930.

Before inoculation, branches were surface sterilized, and washed with sterile water. Three inoculated branches developed a total of 78 infected leaves. Unfortunately, however, check branches developed 17 infected leaves, though the latter occupied a position in most cases in close proximity to inoculated branches, thus indicating a spread of infection from the inoculated branches to the checks.

In the summer of 1931, tags were placed on 50 buds on branches which had showed 100% leaf curl infection earlier in the season. During the following winter isolations were made from these buds at intervals of one month, from October until May.

Among other organisms, *T. deformans** appeared in varying frequency in each series of isolations except those made in February. In the May series, when the organism appeared most frequently, as many as 17 colonies developed in isolations from a single bud.

Infection experiments were again conducted in the spring of 1932 with results essentially similar to those obtained during the previous year.

In September of two successive years suspensions of conidia and atypical spores of *T. deformans* were painted on surface sterilized buds and bark of twigs on sprayed Elberta trees to test the capability of the spores to overwinter on the exposed surfaces. Isolations were made from these twigs at intervals of one month until the following May. *T. deformans* appeared in every series of isolations during both winters.

Various attempts were made in the course of the above experiments to find out whether the conidia, or the atypical spores (resting spores, according

**This organism was compared microscopically with pure cultures of T. deformans obtained from other investigators, and proved similar in every respect.*

to Mix (13)), or both, were overwintering. This was done by making drawings of the spores when they were transferred singly from Petri dishes to tubes where their further development was followed.

Some evidence was obtained to the effect that so far as these experiments were concerned both conidia and atypical spores were overwintering. Many of both types of spores were observed to turn slightly brown in color after remaining on the bark for several weeks.

The evidence obtained in the above experiments on *T. deformans* agrees in general with that obtained by Fitzpatrick (7) who conducted intensive investigations on leaf curl concurrently with the overwintering studies outlined above, and strongly indicates that *T. deformans* overwinters in the form of spores on the surface of buds and bark of *P. persica*.

Coniothyrium sp.

A *Coniothyrium* sp. appeared as a frequent isolant from the surface of buds and bark of *Prunus domestica*, *P. persica*, *P. cerasus*, *P. pennsylvanica*, *Pyrus malus* and *P. communis*. As this organism has been dealt with in a previous article (11) it will not be discussed in detail here. It should be pointed out, however, that in numerous instances this *Coniothyrium* sp. was proved by isolation to overwinter on the above-mentioned hosts in the form of chlamydospores.

Two Members of the Fungi Imperfecti

Two fungi belonging in the Fungi Imperfecti were isolated consistently and frequently each winter from the surface of branches and buds of *P. domestica* in particular, and in some cases from *P. cerasus*. These fungi presumably were saprophytes but were particularly interesting because of their method of overwintering.

One of these fungi was *Torula*-like in character and was referred to that group, but owing to the unsatisfactory situation existing in the taxonomy of this group at the present time, it was impossible to identify it further.

On potato-dextrose agar this organism produced scarcely any mycelium, colonies consisting entirely of elevated, black, compact but not hard, somewhat sclerotium-like and irregularly shaped bodies (Plate III, Fig. 4). Colonies increased rapidly in size both outwards and upwards, frequently reaching the top of the Petri dish or test tube within two weeks. The black masses which composed the colonies consisted almost entirely of groups of dark colored spores, many of which were thick-walled and two-celled. The latter were always borne irregularly in groups on short strands of mycelium which varied considerably both in appearance and length. Groups of spores similar in every respect to those produced in culture were observed on numerous occasions in suspensions of materials removed from the surface of buds and bark of *P. domestica* during the winter.

It should be mentioned in this connection that owing to the sparse development of mycelium and also to the mycological situation referred to above, it was impossible to determine whether the spores produced by this organism should be termed conidia or chlamydospores.

Another fungus, also a member of the Imperfecti, was consistently isolated from sources similar to those from which the *Torula*-like fungus was obtained. It was impossible to identify this organism (which was isolated on 32 occasions from definite monochlamydospores) either on the host or in culture, because on both types of substratum the chlamydospore was the only type of spore produced. On malt agar, potato-dextrose agar and on sterilized cereals of various kinds the organism always produced an abundance of chlamydospores. On potato-dextrose agar the organism developed a dark green, elevated colony, the surface of which was covered with a mat of short, straight hyphae, pointing outwards. The organism grew very slowly in culture, attaining a diameter of approximately 20 mm. in 33 days. In order to isolate it during the winter it was always found necessary to transfer the chlamydospores to other media soon after plating, otherwise they became completely overrun with more rapidly growing organisms.

In older cultures the chlamydospores of this fungus readily separated from the parent mycelium. The chlamydospores were darker in color than the mycelium, had extremely thick walls, occupied either an intercalary or terminal position on the mycelium, contained numerous oil globules, and were usually two-celled in older cultures (Plate III, Fig. 6).

Cladosporium and *Hormodendrum*

Members of both the genera *Cladosporium* and *Hormodendrum* appeared consistently and frequently in isolations from numerous hosts. As distinguished by their morphological characters on potato-dextrose agar, three strains of the former and at least two of the latter were isolated consistently. Presumably all were saprophytes. An examination of the literature dealing with the description and occurrence of members of both of the abovementioned genera would allow all strains of *Cladosporium* isolated to be called *Cladosporium herbarum* and similarly, all strains of *Hormodendrum* to be called *Hormodendrum cladosporioides*. However, since both of the above species names have been applied to most of the saprophytic members of their respective genera, dangerously wide limits have been set up for both species, as is evident in the opinion (1) that one of these species is merely a stage of the other. It was thought preferable, therefore, to refer to them only generically.

As they are of special interest in the present article only because of their method of overwintering, their occurrence is listed in Table II.

TABLE II

SUMMARY OF ISOLATIONS OF *Cladosporium* AND *Hormodendrum* FROM VARIOUS SUBSTRATA

Date	Hosts	Source of isolation	Number of isolants	
			Cladosporium	*Hormodendrum*
Nov.–Mar. (1929-33)	*Prunus domestica*	Monochlamydospores from surface of buds and twigs	15	8
"	*P. cerasus*	"	7	6
"	*P. virginiana*	"	5	9
"	*P. pennsylvanica*	"	12	7
"	*P. persica*	"	7	12
"	*Pyrus malus*	"	3	3
"	Various *Prunus* spp.	Undetermined	64	181

It will be observed from an examination of Table II that both *Cladosporium* and *Hormodendrum* appeared frequently in isolations from the buds and bark of numerous hosts. It will also be noted that *Cladosporium* appeared in all, 49 times, and *Hormodendrum* 45 times in isolations from monochlamydospores.

All strains of *Cladosporium* produced chlamydospores in pure cultures on potato-dextrose agar, and one strain, designated *A*, which was readily distinguished from others by its large verrucose spores, usually developed sclerotium-like bodies as well (Plate III, Figs. 1 and 5).

The evidence outlined above strongly suggests that at least certain members of the genera *Cladosporium* and *Hormodendrum* overwinter on the bud and bark surfaces of many fruit trees in the form of chlamydospores.

Fumago sp.

A species of *Fumago*, similar in many respects to *Fumago vagans* investigated by Fant (5), in 1928, appeared consistently and frequently in isolations from *Prunus domestica*, *P. persica*, *P. cerasus*, *P. americana*, *P. virginiana*, *P. pennsylvanica*, *Pyrus malus* and *P. communis*. The source of these isolations was proved in numerous instances to be chlamydospores. On moist filter paper and on potato-dextrose agar in Petri dish cultures which were either gradually desiccated or which contained numerous other organisms, an abundance of chlamydospores was frequently produced by the *Fumago* sp.

Other Fungi

Numerous other fungi, many of the budding type, were frequently isolated from the hosts already mentioned, but as they were all presumed to be saprophytes no investigation of them was undertaken.

Chlamydospores in Fungi

From the standpoint of etymology the term chlamydospore (chlamyd— combining forms from Greek χλαμύς , χλαμύδος , mantle, chlamys) signifies a spore surrounded by a mantle. Since the earlier days of mycology the term has been employed by Van Tieghem and Le Monnier (19), de Bary (4), and others, in referring to bodies which they regard as accessory

conidia. However, definitions of chlamydospores have been many and varied, and until recent years, the term has been applied to almost any spore which has thick walls and functions as a resting spore, with no consideration of the morphology of the spores concerned. Instances of the occurrence of chlamydospores throughout almost the complete taxonomic range of fungi have been reported.

In the Phycomycetes, Fitzpatrick (6) points out that chlamydospores occur chiefly on old thalli and are not homologous with the sporangia. He also states that they function as resting spores, germinate by germ tubes, are non-deciduous, and may occupy a terminal or intercalary position on the mycelium.

Some confusion has apparently arisen through the application of the term chlamydospore to the azygospores formed by certain members of the Mucorales. But until an intensive investigation of the morphology of bodies commonly referred to as chlamydospores in the various fungi is undertaken, it will be impossible to definitely determine the status of bodies such as azygospores. However, the bodies in certain of the Mucorales designated gemmae by Gaümann (8) and chlamydospores by Fitzpatrick (6) are similar in every respect to those bodies in the Ascomycetes and Fungi Imperfecti which are conceded to be chlamydospores. Therefore, in the case of these Mucorales, it would seem preferable to restrict the use of the term chlamydospore to the thick-walled spore formed within the vegetative coenocytic mycelium, and to exclude azygospores, which are borne on specialized hyphae.

Among the Ascomycetes and Fungi Imperfecti chlamydospores have been reported for most genera. From the standpoint of the present investigations, chlamydospores are of interest chiefly in connection with these two groups of fungi, where the conception of a chlamydospore, at least during recent years, has been fairly well restricted to the limits already defined in this article. However, this definition is not satisfactory for the chlamydospores occurring in certain members of the above groups, not included in the present investigation. For instance, it would exclude chlamydospores formed either endogenously in the conidia of certain species of *Fusarium* and *Ramularia*, or on special lateral branches in certain species of *Fusarium* (18) or in *Thielavia basicola* (3). The use of the term chlamydospore has been confused in certain members of the the Taphrinales (*e.g. T. deformans*) when it has been applied (8) to the sub-epidermal layer of thick-walled cells from which the asci arise. This confusion has arisen, no doubt, because these fungi have been considered by certain mycologists to be closely related to the Protomycetaceae.

Even in recent years the resting spores of the Ustilaginales have frequently been referred to as chlamydospores, but of late there appears to be a tendency on the part of many investigators to avoid the use of the term in connection with the smut fungi. If chlamydospores were considered in the light of function only, to the entire disregard of their morphology, it would then be correct to term a smut spore a chlamydospore. It would also be

equally correct to apply the term to an aeciospore, a urediniospore or a teliospore of a rust fungus as Brefeld (2) and others have in the past considered them. A similar argument would hold even for the zygospore of a Zygomycete. In recent years, however, the restriction of the occurrence of chlamydospores to the vegetative phase and their consequent asexual nature, have been considered to be just as significant regarding their designation as the function which they perform. Chlamydospores have also been reported in certain other Basidiomycetes, but their occurrence in this group will not be discussed in detail.

In the case of certain fungi, members of the Fungi Imperfecti in particular, it may be difficult to distinguish a chlamydospore from a conidium. Such difficulty has been experienced, as pointed out previously, in connection with the *Torula*-like fungus isolated frequently during the present investigations. It is quite true that chlamydospores may tend in one extreme towards sclerotia and in the opposite towards conidia or, more often, towards ordinary vegetative mycelium.

In conclusion it should be mentioned that the secondary importance usually attached to chlamydospores can be traced largely to their lack of diagnostic value from the mycological standpoint. Chlamydospores produced by a single organism vary so greatly both in shape and size that it has in most cases proved impossible to diagnose species by the morphological characters of their chlamydospores.

Sherbakoff (18) however, discovered that the genus *Fusarium* could be delimited into sections on the basis of the presence or absence of chlamydospores; in one case he even distinguished two species of *Fusarium* by their position on the hyphae. Undoubtedly similar situations will be found to exist within other genera as they become more intensively investigated.

The above paragraphs point out the almost universal occurrence of chlamydospores in fungi, as well as a certain confusion regarding the use of the term. The importance of the chlamydospore from the pathological standpoint, which has hitherto been incompletely appreciated, because of its unsatisfactory mycological status, has been emphatically demonstrated by the present, somewhat limited investigations. Future work along similar lines will undoubtedly disclose a similar degree of importance for chlamydospores in numerous other fungi.

Discussion

The present investigations on the overwintering of certain fungi have revealed several new and important features in the life histories of the organisms concerned.

In the case of *Dibotryon morbosum*, the cause of black knot, not only have conidia been found to remain viable on the host throughout the winter, but also a new overwintering stage in the form of chlamydospores has been definitely proved. In cultural studies the interesting discovery was made that *D. morbosum* produced chlamydospores *only* when grown in close prox-

imity to certain other organisms or when chemicals were introduced into the culture medium. Chlamydospore formation was also induced on twigs of *Prunus domestica* when conidia of *D. morbosum* germinated in intimate association with antagonistic bacteria originally isolated from the same host. Though a survey of the literature relevant to the occurrence of chlamydospores in fungi has not revealed data similar to those outlined in the present investigation regarding *D. morbosum*, Porter (14) reports a somewhat similar phenomenon in his investigations on association of organisms.

This phenomenon of chlamydospore formation as observed for the black knot organism may be significant from the standpoint of biologic control. Sanford and Broadfoot (15) discovered that certain fungi and bacteria "suppressed" the pathogenicity of *Ophiobolus graminis*. Numerous other instances of a similar nature have also been reported during recent years, particularly in connection with soil fungi. No investigations are reported, however, which were undertaken primarily to determine the actual fate of the organism, the pathogenicity of which was inhibited by the antagonistic organism(s). It has been shown in the present investigations that the continued presence of the antagonistic organism inhibits the vegetative growth of *D. morbosum* and induces profuse chlamydospore formation on the part of the latter organism. On the removal of the antagonistic organism however, the chlamydospores germinate and the fungus resumes vegetative growth. It is quite possible that similar phenomena may occur in the case of other fungi, particularly soil fungi, because of the continually changing flora consequent upon variations in environmental conditions.

The present investigations, confirming those of Keitt (10) in 1917, demonstrate that the chief method of overwintering of *C. carpophilum* is in the form of mycelium occurring in lesions on the superficial tissues of twigs. Histological studies of these lesions have indicated that certain of them resemble lesions on apple twigs caused by *Venturia inaequalis* (described in detail recently by Marsh and Walker (12)). *C. carpophilum*, as in the case of the latter organism, develops secondary lesions during the winter as a result of the migration of the mycelium between the cuticle and the epidermis, into which region the periderm barriers underlying and delimiting the primary lesion do not extend. It was interesting to note that in cases of infection where both primary and secondary lesions developed, the mycelium of *C. carpophilum* overwinters most frequently only in the secondary lesions where it fruits the following summer. The facts, first, that *C. carpophilum* produces an abundance of chlamydospores in culture, and second, that the organism was isolated from chlamydospores removed from the surface of the host during the dormant season, indicate that the fungus also overwinters in nature in the form of chlamydospores.

The isolation of *Taphrina deformans* from the surface of buds and twigs of *Prunus persica*, and the proof that spores of *T. deformans* can overwinter on the above-mentioned organs, confirms Fitzpatrick's (7) recent findings in regard to this organism and is of particular interest in view of the fact that the method of overwintering of this fungus has heretofore remained in doubt.

It is interesting to note that other fungi, presumably saprophytes, representing six different genera, developed from chlamydospores removed from the surface of many hosts during the winter. Since all of these fungi also produced chlamydospores in artificial culture it is reasonable to assume that they do so in nature. The results of these investigations strongly suggest that many more fungi overwinter in the form of chlamydospores than the literature to date indicates. This is not surprising in consideration of the fact that, from the standpoint of taxonomy, plant pathologists and mycologists have regarded the chlamydospore as possessing relatively little value. This situation, as pointed out above, has been largely responsible for the misapplication of the term chlamydospore.

From the economic standpoint the present investigations emphasize the importance of the application of the dormant spray on fruit trees, since it has been demonstrated that both conidia and chlamydospores of several of the important plant pathogenes can overwinter on the exposed bud and bark surfaces.

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THE RELATION BETWEEN AWNS AND YIELD IN SPRING WHEAT[1]

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Abstract

Considerable interest has been aroused over the possible relationships existing between the presence of awns and the grain yield of wheat. The general consensus of opinion is that awned varieties tend to outyield awnletted or awnless varieties. A number of investigations with barley and wheat have shown that the awns play an important role in transpiration, and that their removal results in reduced yields. Genetic studies dealing with the relation between awns and grain yield of wheat have given contradictory evidence. Certain workers have reported positive, others negative, and still others no relationship, existing between the presence of awns and grain yield.

In the present investigation, the problem was studied with a number of F_3 lines of Reward × Caesium, and also with a number of awned and awnletted strains of Marquillo × Marquis-Kanred. In neither case were any significant relationships found to exist between awns and grain yield. The apparently contradictory results obtained by the different investigators suggest that the relation between the two characters differs, depending both upon the material used and the environmental conditions under which it is grown.

Introduction

Considerable interest has been aroused over the possible relationships existing between the presence of awns and the grain yield of wheat, the general consensus of opinion being that awned varieties tend to outyield awnletted or awnless varieties. As a result most wheat breeding programs are stressing the presence of awns as one of the factors contributing to higher yield. If such an association exists, the reason for increase in yield may be due to several factors. It may be that the awns play an important physiologic role in the metabolism of kernel development, or it may be that the factors governing awns are linked with some of the factors influencing grain yield. Ceres, introduced recently by Waldron (16), is an example of a high-yielding awned variety. On the other hand, there are the various awnless Red Bobs selections (Red Bobs No. 222, Early Triumph and Supreme), which are commonly grown in western Canada, and which have been found to possess high-yielding abilities (1, 2, 7, 15).

A review of the investigations on the relation between awns and grain yield shows that certain workers found awnedness to be associated with increased yield (3, 4, 9, 10, 12), others found no association (6, 13), while still others report a decrease in yield with an increase in awnedness (5). The present investigation deals with the relation between awns and grain yield, in the F_3 of Reward × Caesium, and in a number of awned and awnletted Marquillo × Marquis-Kanred strains.

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Materials and Experimental Methods

Materials

The F_3 only, of Reward × Caesium was used in the present study. Reward, C.A.N. 1509*, is a hard red spring wheat, originating from a Marquis × Prelude cross made at the Central Experimental Farm, Ottawa. It is awnletted and of fair yielding ability. Caesium 0.111, C.A.N. 1256, was obtained from Dr. Talanov of the West Siberian Experimental Station in 1928. It is fully awned and high in yielding ability.

The hybrid strains of Marquillo × Marquis-Kanred were introduced from the University of Minnesota in 1928, and selected at the University of Alberta. Certain of these strains are awnletted, while others are fully awned.

Experimental Methods

The F_3 lines of Reward × Caesium were grown at Edmonton in 1933 in 10-ft. rows, 1 ft. apart, with approximately 50 seeds per row. The plant yield of grain in grams was determined on 57 F_3 lines of Reward × Caesium and five rows of each parent. The plants in each row were pulled separately and the heads wrapped in cheesecloth to prevent loss in handling. The border plants at the ends of each row were noted and were not used for yield studies. Each plant was threshed separately and the yield recorded in grams. The average grain yield per plant for each row was calculated.

Rod row trials consisting of quadruplicated three-row plots were conducted on 34 hybrid strains of Marquillo × Marquis-Kanred on stubble in 1930, and on 28 strains on fallow in 1931.

The method used in measuring the independence and association of awns and yield was by contingency tables, in which the two characters are compared. The relationship between the two characters was obtained by calculating χ^2 and determining the P value from Fisher's χ^2 tables (8). In determining P from the tables, Fisher's suggestion for degrees of freedom was used. For example, a 4 × 3-fold table has six degrees of freedom. A P value of 0.05, or less, is necessary before it can be concluded that an association exists. A P value of 0.05 means that any association that exists between the distribution of the two characters might occur by chance in 1 out of 20 cases.

Literature Review

Zoebl and Mikosch, and later Schmid, cited by Hayes (12), stress the importance of awns as organs of transpiration in cereals. Perlitius, also cited by Hayes (12), conducted transpiration studies with awnless and awned varieties of spring and winter wheat, and with barley. His results were in agreement with those of the previous workers mentioned. He reports that the awned wheats tended to be plumper and to possess a higher starch content than the awnless wheats.

*C.A.N. = *Canadian Accession Number.*

Grantham (10) studied the yield of a number of awnless and awned winter wheats over a period of years. His results show that the average yield of the awned varieties was 3.1 bu. greater than that of the awnless varieties.

Harlan and Anthony (11) found that the removal of the awns in the case of Hannchen and Manchuria barley resulted in lower yields. This reduction in yield, they state, could not be attributed to any injury to the plant. The awned plants showed a greater activity during the period of starch infiltration. The differences in the ultimate weight of the kernels produced were primarily due to differences in the quantity of starch produced. Whether or not the reduction of transpiration, as the result of the removal of the awns, is sufficient to account for a lower rate of starch formation is a question. However, they conclude that the loss of the awn definitely results in lower yield.

Hayes (12) with Marquis × Preston hybrids found that in each of the generations from the F_3 to the F_5, the bearded families gave a higher average yield than the awnless families. During the favorable seasons of 1918 and 1920, the bearded families yielded between 7 and 8% more than the awnless families, while in the unfavorable season of 1919, the difference in yield was 17% in favor of the bearded families.

Clark (3) studied the effect of awns on yield in the F_2 of Kota × Hard Federation hybrids, grown at St. Paul, Minnesota, and Mandan, North Dakota. At both stations the awned plants outyielded the awnless plants by 11 to 15%, the odds being 9:1. Although these results are not significant, Clark states that they indicate that awns probably have some effect on yield under dry as well as humid conditions. In the F_3 a similar relationship was shown between awns and yield. In this case, however, the results were significant.

Clark, Florell and Hooker (4) studied the relation between awns and yield in the F_2 and F_3 of Bobs × Propo and Hard Federation × Propo crosses at Davis, California. In the F_2 of both crosses there was a decrease in yield with increase in the length and number of awns. This may be due largely to greater shattering in the case of the awned plants. In the F_3, in which shattering did not occur, the average yield tended to increase with awnedness. The authors concluded that when shattering was not a factor, there was a tendency toward increased yields with increased awnedness, under the conditions prevailing at Davis.

Goulden and Neatby (9) studied the association between awning and grain yield in rod row trials of awned and awnless strains of H–44–24 × Marquis. They obtained a P value of 0.0004, which indicates a very high probability of association between the presence of awns and high grain yield.

Moskalenko (13) studied the dependence between awnedness and the "elements of productivity" with winter wheat hybrids. He concluded that during the years of 1922, 1925, 1926 and 1927, there was no genetic relationship between awnedness and the "elements of productivity", under the conditions of the Ukranian steppe.

Clark and Quisenberry (5) report on the relation of awnedness to yield in the F_2 and F_3 of Kota × Marquis, grown at Bozeman, Montana. In both the generations studied, significant differences were obtained, showing that the awnletted plants and strains outyielded the awned plants and strains, in spite of the greater extent of shattering in the awned plants.

Clark, Quisenberry and Powers (6), working with Hope × Hard Federation crosses, found no relationship existing between awnedness and grain yield.

Parker (14) reported an experiment on the effect of awn removal on the yield of Kanred wheat. The awns were removed at the blossoming stage, one week later, and four days before harvest. He concludes that mechanical removal of the awns results in lower yields, the reduction in yield being less as the crop approaches maturity.

Experimental Results

The average grain yield per plant was determined on 57 F_3 lines of Reward × Caesium. The data in Table I give the frequency distribution and the average grain yield for the awnletted, segregating and awned F_3 lines and parental rows.

TABLE I

FREQUENCY DISTRIBUTION OF REWARD × CAESIUM F_3 AWNLETTED, SEGREGATING AND AWNED LINES AND PARENTAL ROWS, FOR AVERAGE GRAIN YIELD, GROWN AT EDMONTON IN 1933

Class	Grain yield, gm.										Average yield, gm.
	5.8	6.3	6.8	7.3	7.8	8.3	8.8	9.3	9.8	10.3	
Awnletted				5	2	3			1		7.9 ± 0.15
Segregating	1	1	5	6	4	5	4	3			7.7 ± 0.13
Awned			1	2	5	3	2	2	1	1	8.3 ± 0.15
Reward		2	2	1							6.7 ± 0.07
Caesium								1	1	3	10.5 ± 0.10

NOTE: $\chi^2 = 17.20$, $P = 0.50$.

The data in Table I show that Caesium, the awned parent, gave significantly higher yields than Reward, the awnletted parent. Although the average grain yield of the awned hybrid lines was 0.4 gm. more than that of the awnletted hybrid lines, the difference is not significant in the light of a P value of 0.5. Tests of association and independence were also made on the individual plants for each of the segregating F_3 lines. No significant relationship was found between awns and yield in any of the 29 segregating lines. The results of this experiment indicate that the presence of awns does not significantly increase the yielding capacity of the plants in F_3 lines of Reward × Caesium crosses.

Tests of association and independence were calculated to determine whether or not any relationship existed between awns and yield for the strains of Marquillo × Marquis-Kanred for the two years 1930 and 1931.

The data in Table II show that the average yield of the awnletted strains was 1.9 and 0.5 bu. higher than that of the awned strains in 1930 and 1931 respectively. These differences, in the light of their P values of 0.34 and 0.96 respectively, are not significant. The results of this experiment support those obtained from the F_3 of Reward × Caesium.

TABLE II

AVERAGE YIELD AND P VALUE OF AWNLETTED AND AWNED STRAINS OF MARQUILLO × MARQUIS-KANRED, GROWN ON STUBBLE IN 1930 AND ON FALLOW IN 1931, AT EDMONTON

Class	1930		1931	
	Average yield, gm.	P value	Average yield, gm.	P value
Awnletted	25.1 ± 0.54	0.34	51.7 ± 0.95	0.96
Awned	23.2 ± 0.31		51.2 ± 0.63	

Discussion and Conclusions

The results reported in this paper showing that there is no relation between grain yield and the presence of awns are in agreement with the work of Moskalenko (13) and Clark, Quisenberry and Powers (6). Grantham (10), Hayes (12), Clark (3), Clark, Florell and Hooker (4) and Goulden and Neatby (9) report that the presence of awns is associated with an increase in grain yield. Clark and Quisenberry (5) report a negative relation between the presence of awns and grain yield. These apparently contradictory conclusions of the different investigators indicate that the relation between awns and grain yield differs, depending both upon the varieties used and the environmental conditions under which the material is grown.

From the data reported in this paper, it may be concluded that, under Edmonton conditions, and for the material studied, there is no significant relation between the presence of awns and grain yield.

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A KARYOSYSTEMATIC INVESTIGATION IN THE GRAMINEAE[1]

By A. W. S. Hunter[2]

Abstract

The history and the present state of the classification of the Gramineae are briefly reviewed and a number of the different characteristics on which phylogenetic systems have been based are considered. The subjects of chromosome morphology and the application of idiograms and karyotypes to taxonomic studies are discussed. Avdulov's recently reported findings on the phylogeny of the grasses are summarized and compared with the results of other workers and those obtained in the present investigation. Three species of bamboos were studied for the first time and evidence secured to indicate that the basic number of the tribe is probably not 12 as has been elsewhere reported. In the Festuceae the chromosome number of *Phragmites communis* Trin. was definitely ascertained, confirming Avdulov's supposition that the basic number for the genus is 12. The other three species investigated agreed with the arrangement as proposed by Avdulov. The tribe Chlorideae, with the exception of the genus *Beckmannia*, has been reported to be almost entirely Panicoid with respect to chromosome morphology. This was confirmed in the four species examined. Avdulov's rearrangement of the tribe Hordeae was somewhat altered and a confusion in the nomenclature of the genus *Lepturus* was corrected. An anomalous situation was cleared up in the tribe Agrostideae by the establishment of the chromosome number of *Sporobolus tennuissimus* Kuntz as 40. The specimen of *Anthoxanthum odoratum* L. (tribe Phalarideae) examined provided a very interesting example of secondary splitting in somatic chromosomes. One species was examined in the tribe Melinideae and six in the tribe Paniceae. In the latter tribe no difference could be detected between the several subdivisions of the genus *Panicum*. The same condition held for the large genus *Andropogon* in the tribe Andropogoneae. In the tribe Maydeae the chromosome number of *Tripsacum dactyloides* L. was found to be 9, and the suggestion was made that it may be a link, along with the genus *Coix*, between the Andropogoneae and the Maydeae. The other four species examined all had a basic number of 10.

Introduction

The classification of the family Gramineae has long presented many difficulties to the systematist. Numerous workers have attempted to divide the family in a natural manner, but so far they have failed to reach agreement even on the division into the main groups—subfamilies and tribes.

Brown (7) was the first to recognize the two main subfamilies, which he called "tribes," the Panicoideae and Pooideae. Following him, Kunth (26), Endlicher (14) and Steudel (47) proposed classifications differing from one another only in details and in the number of genera included. They did not, however, recognize the division of the family into subfamilies as proposed by Brown (7), and it was not until the work of Bentham and Hooker (5) that this distinction was revived. Bentham and Hooker called the subfamilies Paniceae and Poaceae respectively. The system of Hackel (16) differed very little from that of Bentham and Hooker (5). He replaced the names of the subfamilies by the terms "Series A" and "Series B" and changed the order of some of the tribes. However, inasmuch as the arrangement of the tribes

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in any linear phylogenetic series is impossible, this difference is not very important. The system is still followed more or less closely by most modern workers. It has been added to and modified mostly by Stapf (quoted by Bews (6)) who has elevated many subtribes to the rank of tribes, and has also split up some of Hackel's larger genera, such as *Andropogon*, changing many sub-genera of earlier authors following Hackel to genera, and creating many new ones. The same has been done for other genera such as *Panicum*, by Hitchcock and Chase (21). Apart from this, Hitchcock and the other American botanists generally follow Hackel in their delimitation of the tribes, though not in their order of arrangement. Bews (6), whose system is followed in this study, draws upon the work of all these authors. He divides the Gramineae into two subfamilies—the Pooideae and the Panicoideae. The Pooideae are in turn divided into the tribes Bambuseae, Phareae, Festuceae, Aveneae, Chlorideae, Hordeae, Agrostideae, Zoysieae, Phalarideae, Arundinelleae and Oryzeae. The Panicoideae are divided into the Melinideae, Paniceae, Andropogoneae and Maydeae.

The earliest classifications were based on external morphology alone. In 1858, however, Trecul (50) noted the organization of the starch grains in plants and classified them as simple and compound. In 1881-82 Harz (17) used these data for building up a phylogenetic scheme for the family. It has since been shown, however, that the type of starch grain in a plant cannot be determined from an examination of its structure in mature seeds but only by a study of its ontogeny. The taxonomic value of this characteristic must therefore be somewhat limited until such time as careful studies shall have been made.

Duval-Jouve (12, 13), Grob (Prat (41)) and Pée-Laby (39) studied the anatomy of the leaves and arrangement of cell types in the epidermis. Pée-Laby (39) was able to distinguish between two anatomically distinct groups of grasses by the arrangement of the chlorenchyma tissue. One group has the chlorenchyma localized in two layers of cells around the vascular bundles. Avdulov (1) calls this arrangement "type 1." The other group has the chlorenchyma uniformly distributed throughout the leaf and is referred to as "type 2." Prat (41) extended the work of Duval-Jouve and Grob. He based his classification not on any one or a few separate characteristics, but on a complete analysis of the size, shape, structure distribution and maturity of the various cell types on all parts of the plant, stem, leaves, glumes, etc. He found that certain characteristics serve to distinguish large groups; others define more restricted groups, and still others the simple forms caused by variation in environment, etc. By this method Prat was particularly successful in studying and amending the classification of the tribe Hordeae, and in indicating its relationship with the rest of the Gramineae.

S. Nawaschin was probably a pioneer in the study of chromosome morphology, but since he published his results in Russian they were available only to a very limited circle of investigators and it was not until 1924, when he published in German (37) that his results became generally known. Lewitsky (30) quotes Nawaschin as saying in 1910 that according to his observations "the chromosomes of *Fritillaria tenella* are distinctly double and distinctly divided in the place where the bundles of the spindle fibers are attached." A year later he distinguished between three types of bivalent chromosomes according to the position of the attachment constriction: (i) U-shaped, almost equal-armed; (ii) U-shaped, distinctly unequal armed and (iii) hook-shaped, one arm being very small. In 1912 he reported "on the dimorphism of the nuclei in somatic cells of *Galtonia candicans*;" showing the presence of special, minute but perfectly constant appendages attached by means of a thread to two (out of four) medium sized chromosomes characteristic of this plant. He called these appendages "satellites," or in German "trabanten" (the latter term is now more widely used), and noted that during the division of the nucleus they split along with the remaining body of the chromosome. Here, for the first time, was the possibility of distinguishing between chromosomes by the peculiarities of their structure. In 1915 Delaunay (Lewitsky (30)) definitely proved the existence of tri-articulate chromosomes. Delaunay claimed that the number of segments and degree of differentiation were characteristic of definite chromosome types of definite species. This character represents the second type of secondary differentiation of the chromosomes.

In the same year that Nawaschin's German paper (37) appeared, Newton (38) and Taylor (48) published results of investigations from which it was evident that they had arrived at similar conclusions.

Sakamura (45) made extensive use of artificial methods to intensify the morphological differentiation of the chromosomes. He came to the conclusion that the presence of constant constrictions in the chromosomes is a general phenomenon in plants and animals, and that at the point of attachment of the spindle fibres the chromosomes always show a constriction or a rudiment of one.

Early observation convinced the investigators that there existed a more or less definite constancy of chromosome number. Further work, however, resulted in an appreciation of the fundamental constancy of the morphological composition of the nucleus. This later became expressed in the form of the "idiogram." Changes of the idiogram in a series of successive species afforded material for drawing up particular phylogenetic lines as well as for establishing some facts in connection with the evolution of the species.

Lewitsky (31) discusses fully this question of the use of karyotypic characters in systematics. He defines an idiogram as the graphic representation of a chromosome set with all the diversity of its structure. Separate species of a genus are characterized by quite definite and constant idiograms. These idiograms, although varying somewhat from one species to another usually maintain the same general type throughout the genus. On the contrary, when passing from one genus to another, the type of idiogram undergoes a complete transformation and appears as a new "karyotype." It would seem, therefore, that in classifying plants all that would have to be done would be to group those species having similar idiograms into the same genus, and into different genera when the karyotype changed. The question, however, is not quite as simple as this, for we are immediately faced with the difficulty of deciding when a difference in idiogram is great enough to justify the establishment of a new karyotype. This is a very difficult point, especially so when it is known that there exists a uniformity in the number and main characteristics of chromosomes through whole groups of genera which, though allied, are yet unquestionably different. Instances of a real coincidence of idiogram for whole large families have been known. On the other hand there may be a clear divergence of idiograms in adjacent species, which are then characterized by different karyotypes.

Lewitsky (31), in his analysis of the differences to be found in idiograms, notes that in general, chromosomes with equal arms indicate a more primitive stage of development, both morphologically and cytologically, than those with sharply unequal arms. Exceptions to this rule occur sometimes in the case of the formation of heads on chromosome fragments in X-rayed plants (32). It should be noticed, however, that the extent to which the constrictions so formed are functional has not yet been finally settled.

Distinction must be made, in the comparison of idiograms, between chromosome sets which have been differentiated through a process of gradual shortening and those which have undergone more complicated processes such as translocation, fragmentation, constriction-shifting, etc. Lewitsky and Araratian (32) conclude that the sharp differences sometimes observed in the idiograms of closely related species may be due to fragmentation and subsequent loss of portions of the chromosomes, while close similarity of idiograms in morphologically dissimilar genera indicates that the differences between the genera are chiefly genic. Another explanation for the wide difference in idiograms sometimes noticed in related species is afforded by the translocation of parts of chromosomes following fragmentation. Lewitsky and Araratian (32) cite an instance of such a change in a triploid *Crepis*. Similar transformations have been produced by the action of X-rays.

Thus, though the taxonomic significance of one or another of the karyotypic peculiarities is, generally speaking, variable, nevertheless some of them characterize large systematic units and others small ones. In this respect the first place is taken by the basic number and the size of the chromosomes.

A basic number for a polyploid series is usually of great systematic importance, characterizing whole families and tribes. The size of the chromosome is used to separate, for example, subfamilies in the Gramineae and tribes in the Leguminosae, as well as many genera, species and varieties. Next in order of importance come idiogram types, characterized by changes of greater or lesser degree in the length and structure of the arms of the individual chromosomes. Of least importance is the polyploid multiplication of whole chromosome sets.

In conclusion, Lewitsky and Araratian (32) state that "the idea of the karyotype as a karyological characteristic especially and exclusively applied to generic definitions or to any other definite systematic unit does not hold. One cannot state what extent of similarity of idiogram involves their subordination to the same karyotype. On the ground of larger or lesser community of characters, one may distinguish in one and the same complex of forms karyotypes of families, genera, species and races. Therefore, the karyotype is a karyological characteristic of a group of allied forms which may be applied to any systematic unit."

Smith (46) has furnished a striking example of the significance of chromosome morphology in relation to systematics. His arrangement of the Anchuseae on this basis alone accords perfectly with the revision of the group made by Johnston (Smith (46)) from a general taxonomic study.

Early attempts to classify the Gramineae on a karyological basis met with little success, however, on account of the lack of a sufficiently broad collection of material and the use of meiotic instead of mitotic chromosomes, together with limited concepts of chromosome morphology. Meiotic chromosomes rarely retain their individuality and they are of very limited value for the purpose of karyo-systematics.

Avdulov (1) was the first to realize and correct these faults and in consequence was able to propose a system of classification which is, in the main, in accordance with recent findings from other fields of study.

Materials and Methods

The material used was collected by Dr. C. L. Huskins in 1930 while at the John Innes Horticultural Institution, Merton, England. In 1931 Avdulov's (1) important and extensive study on the karyo-systematics of the Gramiueae was published. This research has resolved itself into a scrutiny and extension of his work. The figures are from root-tip preparations fixed in LaCour's (28) 2BE or 2BD fixatives. Sections were cut 14μ thick and stained by LaCour's chromic acid modification of Newton's gentian violet method. Drawings were made with the aid of a camera lucida, 30 $\times$ ocular and Zeiss objective 1.5 mm., N.A. 1.3, giving a magnification at table level of approximately 6,900$\times$. This has been reduced in reproduction to approximately 3,450$\times$.

Table I shows a summary of the classification and somatic chromosome numbers of the species examined in the present investigation. The classification, as mentioned, is based on Bews' (6) system.

TABLE I

LIST OF SPECIES EXAMINED AND THEIR SOMATIC CHROMOSOME NUMBER

Subfamily Pooideae	Chromosome number	Subfamily Panicoideae	Chromosome number
Bambuseae		Melinideae	
Arundinaria fortunei Fenzi.	48	*Melinis minutiflora* Beauv.	36
Arundaria pygmaea Kurz.	54	Paniceae	
Phyllostachys flexuosa	54	*Digitaria exilis* Stapf.	54
Festuceae		*Echinochloa frumentacea* (Roxb.) Link.	36
Arundo donax L.	110	*Oplismenus burmanii* (Retz.) Beauv.	72
Phragmites communis Trin.	48	*Brachiaria mutica* Stapf.	36
Gynerium argentea (Nees) Stapf.	76	*Pennisetum clandestinum* Hochst.	36
Melica altissima L.	18	*Cenchrus tribuloides* L.	34
Chlorideae		Andropogoneae	
Eleusine coracana Gaertn.	36	*Miscanthus saccharifera* Benth.	64
Chloris gayana Kunth	20	*Miscanthus sinensis* Anderss.	42
Eustachys sp.	40	*Andropogon eliottii* Chapm.	20
Cynodon dactylon Pers.	30	*Andropogon scoparius* Michx.	40
Hordeae		*Apluda mutica* L.	20
Lepturus cylindricus Trin.	52	Maydeae	
Agrostideae		*Tripsacum dactyloides* L.	36
Sporobolus tennuissimus Kuntze.	40	*Coix lachryma-jobi* L.	20
Phleum phleoides L.	14	*Euchlaena mexicana* Schrad.	20
Phalarideae		*Euchlaena perennis* Hitch.	40
Anthoxanthum odoratum L.	20	*Zea mays* L. subsp. *indentata*	20
Phalaris arundinacea L.	28		

Observations

Subfamily Pooideae

Bews (6) describes the subfamily Pooideae as follows: "Spikelets two to many flowered or reduced from above downward to a one flowered condition, with or without one or more empty lemmas, or a prolongation of the rachilla above the single fertile floret. Sometimes there may be empty lemmas below the fertile floret, usually two in number. The mature spikelets break up, leaving the glumes behind, or if they fall off entire with the glumes, then they do not consist of two heteromorphous florets as in the Panicoideae. The spikelets are usually more or less laterally compressed. The basic chromosome number in a great many is seven."

Tribe Bambuseae

This tribe is characterized by an extraordinary variety in the reproductive organs, yet despite this diversity it forms throughout a natural group which is more readily separated from its neighbors than any of the other tribes. The connecting character is chiefly a vegetative one, the culm being lignified either throughout its entire length or only at the base. The sessile leaves are also a general characteristic.

The internal anatomy has not been investigated to any very great extent. Prat (41) maintains that the epidermis of such members of the Bambuseae as he has analysed resembles more closely the epidermis of the Panicoideae than it does the epidermis of the Pooideae. Certain anatomical and morphological peculiarities, however, separate them from the former and he considers it better to create a new subfamily, the Bambusoideae, for the tribe Bambuseae.

Cytological data are scarce. Avdulov (1) examined an undetermined species from each of the genera *Bambusa*, *Arundinaria* and *Phyllostachys*. In each case he found the chromosome number to be somewhere between 68 and 74. More recently, Yamaura (51) reported 48 as the diploid number for *Sasa kozasa* Nakai, *Sasa kurilensis* var. *cernua* Nakai, and var. *Uchidai* Makino, *Sasa paniculata* var. *paniculata* Nakai, *Sasamorpha borealis* Nakai and *Pleioblastus maximowiczii* Nakai, and 72 as the diploid number for *Bambusa nana* Roxb. The present investigation included three species. Their chromosome numbers were as follows: *Arundinaria fortunei* Fenzi, $2n=48$ (Fig. 1); *Arundinaria pygmaea* Kurz., $2n=54$ (Fig. 2); *Phyllostachys flexuosa*, $2n=54$ (Fig. 3).

Yamaura (51) considers the basic number of the Bambuseae to be 12, but this is rendered doubtful by results here published unless great differentiation of chromosome number has occurred within the tribe. This question of basic number is associated with that of the primitiveness of the tribe. Certain features of the flower of the bamboos have led systematists to believe that they are not far removed from the original ancestors of the grasses. Lamb (29) placed the tribe at the tip of the phylogenetic tree of the family, but Schellenberg and also Hayek (Avdulov (1)) considered it as a side branch of development, that is, as derived from the same ancestor as the rest of the grasses. Prat (41) notes that the flowers of the Bambuseae approach closer to the typical monocotyledon flower than any of the other grasses, and that certain peculiar characteristics of the epidermis are found also in the Cyperaceae. Evolution in the epidermis of the Gramineae seems to have been in the direction of a progressive simplification, and it is interesting to note that in epidermal elements the Bambuseae are the most variable and the most complex of all the grasses.

Avdulov (1) considers that the basic number in the Gramineae has undergone a progressive diminution, probably from some such number as 12 to the 7, 6 and even 5, as found in the Pooideae and that this reduction in the basic number was preceded by an increase in size of the chromosomes. Lewitsky (30) maintains that in general, small chromosomes with equal arms indicate a more primitive stage of development, both morphologically and cytologically, than those with sharply unequal arms. This primitive morphological condition is well indicated in the chromosomes of the species here illustrated and also in those reproduced by Avdulov (1) and Yamaura (51).

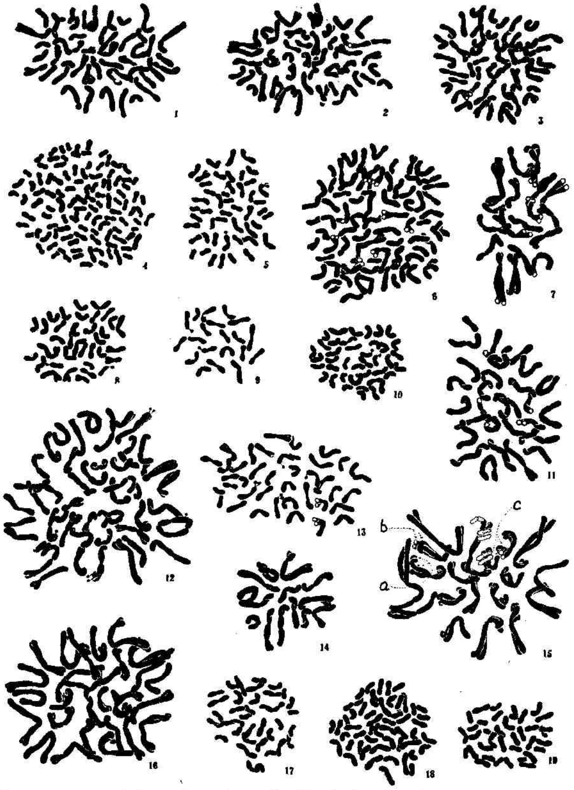

FIG. 1. *Arundinaria fortunei Fenzi*, $2n = 48$. FIG. 2. *Arundinaria pygmaea Kurz.*, $2n = 54$. FIG. 3. *Phyllostachys flexuosa*, $2n = 54$. FIG. 4. *Arundo donax L.*, $2n = 110$. FIG. 5. *Phragmites communis Trin.*, $2n = 48$. FIG. 6. *Gynerium argentea (Nees) Stapf.*, $2n = 76$. FIG. 7. *Melica altissima L.*, $2n = 18$. FIG. 8. *Eleusine coracana Gaertn.*, $2n = 36$. FIG. 9. *Chloris gayana Kunth*, $2n = 20$. FIG. 10. *Eustachys sp.*, $2n = 40$. FIG. 11. *Cynodon dactylon Pers.*, $2n = 30$. FIG. 12. *Lepturus cylindricus Trin.*, $2n = 52$. FIG. 13. *Sporobolus tennuissimus Kuntze*, $2n = 40$. FIG. 14. *Phleum phleoides L.*, $2n = 14$. FIG. 15. *Anthoxanthum odoratum L.*, $2n = 20$. FIG. 16. *Phalaris arundinacea L.*, $2n = 28$. FIG. 17. *Melinis minutiflora Beauv.*, $2n = 36$. FIG. 18. *Digitaria exilis Stapf.*, $2n = 54$. FIG. 19. *Echinochloa frumentacea (Roxb.) Link.*, $2n = 36$.

Tribe Festuceae

Kunth (26) originally set up three tribes instead of the one tribe *Festuceae*, out of which the Bambuseae were later divided off. Bentham and Hooker (5) combined the three main tribes into one and allowed the fourth, Bambuseae, to stand. Hackel (16) adhered to this classification and later, along with Bentham and Hooker (5), divided the tribe into a whole series of subtribes. Much confusion followed as the individual constituents of the tribe were interpolated here and there throughout the system. Finally the view was taken that the whole multiplicity of several-flowered Gramineae, with the exception of certain specially differentiated tribes, the Aveneae and Hordeae, should be classed together as the Festuceae.

Bews (6) considers the Festuceae to be the most primitive of the *common* grasses. This claim to primitiveness is based chiefly on their many-flowered spikelets and short glumes. The tribe is divided into seven subtribes of which the first two, Centothecinae and Arundinae are the most primitive. The main line of evolutionary differentiation commences in the Festucinae and is followed through in the Dactylidinae-Seslerinae group, the Melicinae, the Eragrostinae and the Pappophorinae. The last subtribe is in some respects the most advanced of all the Festuceae though on the whole it is still rather specialized.

Cytologically the tribe may be divided into three groups which by no means agree with the established subtribes. The majority of genera which Avdulov (1) investigated belong to the temperate zones and are marked by large chromosomes having the basic number 7, type 2 leaf anatomy and clumped starch grains. In this first group belong the genera *Glyceria*, *Puccinellia*, *Poa*, *Festuca*, *Sclerochloa*, *Scleropoa*, *Briza*, *Dactylis*, *Desmazeria*, *Lamarckia*, *Sesleria*, *Koeleria*, *Echinaria*, *Melica*, *Catabrosa*, *Bromus*, *Boissiera* and *Schismus*. Polyploidy is observed in the genera *Poa*, *Glyceria* and *Puccinellia*, and a probable instance of the loss of a chromosome pair from a tetraploid form of a series with the basic number 7 is afforded by *Koeleria phleoides* which has 26 chromosomes in comparison with the multiples of seven of the other species investigated.

In the genera *Echinaria*, *Melica* and *Catabrosa* the basic number deviates from the typical 7 of this group, being 9 in the first two and 10 in the last. The specimen of *Melica altissima* here examined (Fig. 7) agrees with Avdulov's (1) material in number and morphology of chromosomes. In *Schismus calicinus* the chromosomes are considerably smaller than usual and the basic number is 6. Inasmuch as none of these differences are bound up with any sharp phenotypic or karyotypic difference, Avdulov does not object to their inclusion in this group.

The second karyo-systematic group contains, so far, only the genera *Diplachne* and *Eragrostis*. The basic number is 10 and the chromosomes are small. These characters of the karyotypic structure are connected with a series of morphological characters. In its cytology, the group is reminiscent of the Panicoideae and the Chlorideae. The resemblance extends to the

epidermis. Prat (41) analyzed the epidermis of *Eragrostis pilosa* and *E. minor* and remarked that even though there is considerable similarity between the epidermal characteristics of this genus and those of the Panicoideae, yet, owing to the complexity and heterogeneity of the Festuceae he does not consider it necessary to remove the genus from that tribe, but merely to give it a special position within the tribe.

Bews (6) notes that Stapf elevated the subtribe Eragrostinae to the rank of a tribe and placed it close to the Panicoideae and also that he removed the genus *Diplachne* to the Chlorideae. As will be seen later this latter tribe is itself very Panicoid in character and there thus exists a remarkable unanimity among three methods of classification; morphological, anatomical and cytological.

The third group has small chromosomes, a basic number 12 and type 2 leaf anatomy. The genera *Centotheca*, *Uniola*, *Phragmites* and *Arundo* fall into this class. The characteristic of small chromosomes in multiples of 12 is common to the groups to which *Oryza* (Oryzeae) and *Erharta* (Phalarideae) belong, and which they also resemble in structure of starch grains, leaf anatomy and geographical distribution.

In the present investigation, *Phragmites communis* (Fig. 5) was found to have 48 chromosomes, agreeing with Avdulov's (1) belief that the basic number for the genus is 12. The genus *Arundo* is closely allied to *Phragmites* and *A. donax* (Fig. 4) was found to have 110 chromosomes.

The genus *Gynerium* is morphologically closely related to *Centotheca*, *Arundo* and *Phragmites* and the chromosomes of *Gynerium argentea* (Fig. 6) though different in number, $2n = 76$, are very similar to those of the species of *Centotheca* and *Uniola* which Avdulov (1) described. Recently, Avdulov (3) published 72 as the somatic number of an undetermined species of *Gynerium*. In the light of this it is possible that some of the extremely small bodies considered as chromosomes in the present material may be only fragments and the basic number of the genus be actually 12 as in the rest of the group.

Tribe Aveneae

In the tribe Aveneae "the glumes are lengthened, and, with rare exceptions, exceed the nearest lemma, often exceeding the whole spikelet, which is thus enclosed and protected by the enveloping glumes. The lemmas remain primitive in being usually 5 to many nerved, and are often awned from the back or sometimes from the tip, or between teeth or lobes, or more rarely are awnless. The spikelets, as in the Festuceae, are two or more flowered, very rarely they are single flowered." (Bews (6).)

The tribe has been regarded by most authors as one of the most natural, yet there are very marked differences within it which may interfere with this naturalness. The position of the awn on the lemma divides the tribe into two groups which correspond very closely with their geographical distribution. Those genera which have the awn springing from the back of the lemma, or which in a very few cases lack awns, belong to the northern group, native to

the north temperate and cold zones. The remaining awn types are almost exclusively in the southern group, and are found in the northern hemisphere only in tropical regions. In the northern group are the genera *Holcus, Aira, Antinaria, Molineria, Deschampsia, Corynephorus, Ventenata, Trisetum, Arrhenatherum, Avena, Gaudinia* and *Airopsis*, while the southern group includes *Prionachne, Eriachne, Zenkeria, Coelachne, Micrairia, Achneria, Anisopogon, Danthonia* and *Amphibromus*. This southern group is very heterogeneous.

Avdulov's (1) investigation covers only genera from the northern group, *Corynephorus, Holcus, Trisetum, Deschampsia, Arrhenatherum*, and *Avena*, and he finds the size and number of the chromosomes to be quite uniform. The basic number, with one or two unimportant exceptions, is 7. The southern group has not yet been studied cytologically.

There is no doubt that the northern members of the Aveneae are very closely related to the Festuceae and Hordeae. Prat (41) considers the Aveneae and Hordeae as subordinate groups of the tribe Festuceae, the Hordeae being those Festuceae with sessile spikelets and the Aveneae those with dorsally articulated awns. This close relationship is amply supported by such karyological data as are available at present. Whether or not an investigation of the southern group of the Aveneae, those not characterized by dorsal articulation of the awn, will suggest their removal from this large Festuceae group remains to be seen.

Tribe Chlorideae

The Chlorideae are distinguished from other tribes by the presence of two series of closely touching spikelets which form a one sided spike. The differences within the tribe, however, are large.

Bews (6) subdivides the tribe into three groups based on the reduction of the number of flowers in the spikelet. The first group contains those with two to several perfect florets in each spikelet, among which are *Eleusine, Dactyloctenium, Beckmannia* and *Leptochloa*. Some of the genera in this group are hardly distinguishable from the Festuceae.

The members of the second group have only one perfect floret and one or more imperfect florets in each spikelet. *Bouteloua, Gymnopogon, Chloris, Eustachys* and *Munroa* are some of the genera which fall in this class.

The most advanced group contains those genera with the spikelets having definitely one floret, with or without a prolongation of the rachilla. The most important representatives are *Cynodon* and *Spartina*.

The anatomy of the leaves of the Chlorideae differs from that of most of the Pooideae in being type 1. Guntz (1886), quoted by Avdulov (1) placed *Spartina* in type 1, while Duval-Jouve (13) put it in type 2. Avdulov unhesitatingly classifies it as type 1 after inspection of a colleague's material. The single exception to the rule seems to be the genus *Beckmannia* which definitely has type 2 chlorophyll arrangement.

All the genera, with the possible exception of *Gymnopogon*, have clumped starch grains. Grob. (1896; Avdulov (1)) demonstrated the presence of two-celled hairs, "Winkelhaaren," on the epidermis of the Panicoideae and also in a few species of the Pooideae. It is interesting to note that all the Chlorideae with the exception of the genus *Beckmannia* possess these two-celled hairs and that Prat (41) considers the genera *Dactyloctenium*, *Eleusine*, *Spartina*, and *Bouteloua* to resemble more closely the Paniciodeae than they do the Pooideae. The tribe shows the same relationship in geographical distribution, *Beckmannia* being a native of temperate regions while the rest of the tribe is more or less tropical or subtropical as is the majority of the Panicoideae.

Avdulov (1) divides the tribe into two karyo-systematic groups. The first is characterized by small chromosomes and the basic numbers 9, 10 and rarely 12. Into this group fall the majority of genera investigated including *Spartina*, *Cynodon*, *Trichloris*, *Dinebra*, *Eleusine*, *Dactyloctenium*, *Leptochloa*, *Buchloe* and *Bouteloua*. In the present study *Chloris gayana* (Fig. 9) was found to have 20 chromosomes, and an undetermined species of *Eustachys* (Fig. 10) had 40 chromosomes. Hackel considers the genus *Eustachys* Desv. to be a subgenus of *Chloris* Sw. Nash (36) points out, however, that most of the points of resemblance of the two genera are common to the tribe Chlorideae as a whole. The details of the spikelet and geographical distribution, at least in North America, are so different that Nash concludes that they should be considered as separate genera. Bews (6) also treats them in this manner and cytological results appear to favour this system. The two genera have similar types of chromosomes but they are present in very different proportions. This might indicate a close relationship and at the same time a difference, which when coupled with large enough morphological changes, would justify their separation.

The present examination of *Eleusine corcarana* (Fig. 8) gave the same number ($2n = 36$) as reported by Avdulov (1). *Cynodon dactylan* (Fig. 11) however was found to have 30 instead of 36 chromosomes. Avdulov considered the basic number of the genus to be 12 but in the light of the above results this appears to be doubtful. The basic number appears rather to be 10 as in most species of this group.

In the genus *Spartina* Avdulov (1) examined *S. cynasuroides* Willd. and *S. schreberi* J. F. Gmel. In the former he counted from 80 to 88 chromosomal bodies and in the latter 40 to 43. He noticed, however, that if any more than 80 or 40 bodies, respectively, were present then there was a corresponding number of chromosomes without attachment constrictions. He therefore assumes that those chromosomal bodies above 80 or 40 are merely fragments and that the basic number for the genus is 10. Church (10), however, reported 14 as the haploid number for *S. michauxiana* Hitch which Saint-Yves (44) says is synonymous with *S. cynosuroides* Willd. and Huskins (22) in his investigation of the origin of *S. townsendii* found it to have 126 chromosomes,

S. alterniflora to have 70 and *S. stricta* to have 56. Church (10) had reported 14 bivalents and 14 univalents in *S. alterniflora*. There is evidently considerable variation and polyploidy within the species of this genus but multiples of 7 seem most common. Avdulov's (1) *S. cynosuroides*, therefore, probably had 84 chromosomes and his *S. schreberi* 42. All the species so far examined are very similar in regard to the morphology of their somatic chromosomes. They are also similar to the rest of Avdulov's first group in this respect. The similarity is strengthened by a series of morphological and anatomical characteristics and there is therefore no need to remove the genus from this group.

The second group contains as yet only the genus *Beckmannia*. Avdulov (1) examined *B. erucaeformis* Host var. *ramosa* Paczosky and *B. erucaeformis* Host var. *baicalensis* W. Kusnezow, and counted 14 chromosomes in each. The chromosomes are large and altogether resemble those of the northern grasses of the Phalarideae, Aveneae, Festuceae and Agrostideae. Therefore, in addition to leaf anatomy, epidermal hairs and geographical distribution, the genus *Beckmannia* differs from the rest of the tribe in its cytology. These characteristics plus a whole series of morphological characters indicate that the genus would be more properly placed with the tribe Festuceae.

Tribe Hordeae

The Hordeae are still more advanced than the Chlorideae in having the spikelets always definitely sessile and usually more or less sunk in the notches on opposite sides of a spike. With the exception of one isolated genus, *Pariana*, they are more or less confined to the temperate regions. The tribe as a whole is extremely uniform, both from the morphological and from the anatomical point of view. Prat (41, 42) made an exhaustive study of the epidermal characters of a number of the genera and came to the conclusion that with the exception of *Nardus* and *Jouvea*, and two other small genera, the tribe was remarkably homogeneous.

There are several differences within the tribe, however, which serve to break it up into sub-tribes. Harz., 1880–1882, and Hayek, 1925 (quoted by Avdulov (1)), divided the tribe into the Triticeae or Hordeae with simple starch grains, and the Lolieae with compound starch grains. Hackel (16) divided the tribe into six sub-tribes, the Nardeae, Lolieae, Leptureae, Triticeae, Elymeae, and Parianeae. Bews (6) combined some of these and proposed the following classification:—

(i) Parianinae (=Parianeae Hackel) containing the isolated genus *Pariana*.

(ii) Elyminae (=Elymeae Hackel) containing the genera *Elymus*, *Sitanion*, *Hystrix*, *Hordeum* and *Asprella*.

(iii) *Triticinae* (=Lolieae, Leptureae and Triticeae Hackel) containing the genera *Agropyron*, *Haynaldia*, *Heteranthelium*, *Triticum*, *Secale*, *Lolium*, *Kerinozoma*, *Jouvea*, *Scribneria*, *Psilurus*, *Ischnurus*, *Lepturus* and *Pholiurus*.

(iv) Nardinae (=Nardeae, Hackel) containing only the genus *Nardus*.

Prat (42) considers the following subdivision of the tribe to be more natural:—

(i) Triticae (2 glumes; spikelets transverse and solitary): *Agropyron*, *Aegilops*, *Triticum*, *Secale*, and *Haynaldia*.

(ii) Hordinae (2 glumes; spikelets transverse and 2–6 at each node): *Hordeum*, *Elymus* and probably *Hystrix* and *Sitanion*.

(iii) Loliae (1 glume; spikelets longitudinal): *Lolium*, *Lepturus*, *Monerma* and *Psilurus*.

(iv) Pholiureae (2 glumes; transverse spikelets but with deeply excavated rachis and epidermis of paleas very little differentiated): *Pholiurus* and *Scribneria*.

There are two genera now generally included in the Hordeae which have given taxonomists considerable trouble; these are *Nardus* and *Jouvea*. The retention of the genus *Nardus* within the Hordeae is morphologically dependent on the fact that the inflorescence is a simple spike. Numerous anatomical features clearly separate it from that tribe, however, and to these Prat (41) has added a series of epidermal observations. The retention of the genus within the Hordeae destroys the admirable unity of that tribe and the only alternative seems to be to create for it a special tribe. This tribe has been called the Nardoideae. Further investigation reveals that this new tribe does not properly belong in the subfamily Pooideae at all, but in the Panicoideae. Prat (42) also transfers the genus *Jouvea* to the Panicoideae and suggests that the genera *Kralikia* Coss and *Kerinozoma* Stend., likewise belong in that subfamily.

With the exception of *Zea mays*, the common cereals, of which three occur in this tribe, are probably the best known, both cytologically and genetically, of all the grasses. The genus *Triticum* forms a definite polyploid series with 7 as its basic number, and is an important example of the use of chromosome numbers alone in classification. The cytological grouping agrees exactly with the classification based on morphological and serological grounds, as well as on the basis of size of pollen grain, rust resistance and mutual fertility or sterility.

Aegilops, *Hordeum* and *Agropyron* likewise form polyploid series within the genus; *Aegilops* with 7 and 14 and *Hordeum* with 7, 14 and 21 chromosomes. Peto (40) reported an almost complete series from diploid to decaploid in *Agropyron*. He also obtained considerable evidence of hybridity. This would help to explain the intergradation of forms frequently observed in the genus.

Avdulov (1) found three different types of chromosome set within the genus *Agropyron*. The first type of set is composed exclusively of *V*-shaped chromosomes (*A. caninum*, *A. cristatum*, etc.); the second type has every chromosome with one arm reduced to a head *(A. prostratum)*, and the third type is an equal mixture of the first two types *(A. orientale)*. He suggests that *A. orientale* may be the result of a cross between a member of the *V*-chromosome group and a species with only headed chromosomes.

Avdulov studied three species of *Lepturus* and one of *Monerma* and observed the following chromosome numbers:—*Lepturus incurvatus* (L.) Trin. ($2n=36$); *Lepturus filiformis* (Roth.) Trin. ($2n=14$); *Lepturus pannonicus* Kunth ($2n=14$); *Monerma cylindrica* Coss and Dur. ($2n=26$). The specimen corresponding to *M. cylindrica* which was examined in the present investigation (Fig. 12) had 52 chromosomes and is a tetraploid. Camus (8) notes that the name *Lepturus* has priority over *Monerma*, and that therefore *Monerma cylindrica* Coss and Dur. should be called *Lepturus cylindricus* Trin. She also places the three species considered by Avdulov as *Lepturus* in the genus *Pholiurus*. These three species she considers should be named *P. incurvus* A. Camus, *P. filiformis* A. Camus and *P. pannonicus* Trin., respectively.

It is evident from Avdulov's (1) illustrations that the four species mentioned above have chromosomes of similar types but that they have them in different proportions. Their close relationship is therefore reflected cytologically. We appear to have an instance of redistribution of chromatin; such loss or change as has occurred has not had any striking effect on the phenotype.

Avdulov (1) removes these four species to his tribe Festucaceae which is a member of his series Festuciformes and contains most of the Festuceae and Aveneae. He also examined *Nardus stricta* (26 chromosomes), *Lolium italicum* (28 chromosomes) and *Psilurus nardoides* (28 chromosomes) and included them in his Festucaceae. Prat (41) found considerable similarity between the epidermis of the Hordeae, Festuceae and Aveneae, but the inclusion of *Nardus* in this group is absolutely contrary to practically all existing morphological, anatomical and epidermal evidence. As has already been pointed out, this genus is very different from the rest of the tribe and approaches the Chlorideae in its resemblance to the Panicoideae. In spite of the almost entire lack of Panicoid characters (more or less small chromosomes in multiples of 9 or 10) in its karyotype, it nevertheless seems to belong naturally to that subfamily. This is a case where more or less definite karyological evidence must be subordinated to an overwhelming weight of evidence of another nature.

Unfortunately, the genera *Jouvea*, *Kralikia* and *Kerinozoma* have not so far been cytologically examined. The remaining genera fall in Bews' (6) subtribes Elyminae and Triticinae or into Prat's (42) subtribes Triticae and Hordinae. Avdulov (1) finds this to be a very uniform group with chromosomes all more or less the same in size and characterized by the basic number 7. He leaves these genera in their original position in what he calls the tribe Frumentaceae which contains also the genera *Bromus* and *Boissiera* of the Festuceae. Thus, with the exception of the genus *Nardus* his classification agrees exactly with that of Prat (42) and shows the artificiality of grouping the subtribes Lolieae-Leptureae and Triticeae. Such correspondence between classifications based on two so entirely different characters is good evidence of correctness.

Tribe Agrostideae

According to Bews (6), "in the Agrostideae the spikelets are definitely one-flowered, without sterile lemmas or male flowers, so that the usual evolutionary trend seen in the tribes so far dealt with, namely, the reduction of the number of flowers in the spikelet, does not apply to this tribe." Other important evolutionary trends are well illustrated, however, and are used as a basis for subdivision into subtribes. "The most advanced subtribe, the Stipinae, have the lemma indurated, or at least much harder than the glumes at maturity, and closely enveloping the fruit. The others have a hyaline or membranaceous lemma, more delicate than the glumes. These are further subdivided into two other subtribes, the Phleinae with the stigmas having short branches all round and projecting from the apex of the lemmas, and the Agrostinae with the stigmas plumose, projecting from the side of the spikelet."

The Phleinae are a distinct, but not important, subtribe. *Phleum*, *Heleochloa* and *Alopecurus* are the only genera of importance. The genus *Sporobolus* is rather distinct from either the Agrostinae or Phleinae, but is included in the former by Hackel. Stapf (Bews (6)) separates it as a distinct tribe, the Sporoboleae, and places it next the Eragrosteae which he removed from the Festuceae. The subtribe Stipinae is the most advanced of the three as is evident from their more efficient means of seed dispersal and adaptation to a more or less xerophytic habitat.

Cytologically the tribe may be divided into three fundamental groups. The first is represented by the genera *Muhlenbergia* and *Lycurus* on the one hand and *Heleochloa* and *Sporobolus* on the other. It is characterized by small chromosomes and a basic number of 10 for the first part and 9 for the second. Avdulov (1) bases his conclusion as to the basic number of the second part on *Heleochloa schoenoides* which has 36 chromosomes and on two forms of *Sporobolus indicus*, one of which has 18 and the other 36 chromosomes. *S. tennuissimus* (Fig. 13) was examined in the present investigation and found to have 40 chromosomes which gives it the basic number 10 and makes it conform with the first part of the group. The group as a whole resembles the subfamily Panicoideae of Bews, in regard to its leaf anatomy, geographical distribution and structure of the ligule as well as in its cytology. Krause, according to Avdulov (1), demonstrated the morphological connection of *Heleochloa* with *Sporobolus* and of both with the Chlorideae and the genus *Tragus* of the Zoysieae. Both these tribes are similar to this group in cytology, structure of starch grains and leaf anatomy and, as Prat (41) points out, in certain epidermal characteristics also.

The second cytological group contains the genera *Calamagrostis*, *Agrostis*, *Mibora*, *Cornucopiae*, *Phleum*, *Alopecurus* and *Milium*. It is characterized by large chromosomes and the basic number 7, with the exception of *Milium vernale* which has 18 chromosomes. *Milium effusum*, however, has 28 chromosomes. *Phleum phleoides* (Fig. 14) was the only representative of this group examined in the present investigation. It had 14 chromosomes. Avdulov

(1) studied four species and Gregor and Sansome (15) studied two strains each of two species of *Phleum*. The basic number 7 was constant throughout.

Three genera of this group, *viz.*, *Phleum*, *Mibora* and *Alopecurus* are connected morphologically with the genera *Hierochloa*, *Anthoxanthum* and *Phalaris* in the tribe Phalarideae by reason of the fact that the awn springs from the tip of the glume. In *Apera*, however, it arises from the back of the glume as in the Aveneae. The group agrees with the above members of the Phalarideae and with the Aveneae in having clumped starch grains, type 2 leaf anatomy, parallel epidermal organization (Prat (41)), and in being distributed mostly in the northern hemisphere. The similarity extends also to their cytology. All have large chromosomes, and the basic number 7 is characteristic of the Aveneae and two of the three genera of Phalarideae.

The third group, represented by the genera *Stipa* and *Oryzopsis*, is characterized by small chromosomes with a basic number 12. Of the eight species of *Stipa* investigated by Avdulov (1), one, *S. sibirica*, has 24 chromosomes and the rest have 44. These species are presumably tetraploids derived from a diploid form with a basic number 12, which had lost one pair of chromosomes. The two species of *Oryzopsis*, *O. miliacea* and *O. virescens*, both have 24 chromosomes.

Cytologically this group approaches *Oryza* (Oryzeae), where the basic number is also 12 and where the chromosomes are also small. These genera are likewise similar floristically, all approaching a more or less primitive, hexameric type of flower structure. There thus seems to be a linkage between small chromosomes with the basic number 12 and this flower type.

Tribe Phalarideae

This is a small tribe containing only six genera. It is divided naturally into two groups. The first contains *Ehrharta*, *Microlaena* and *Tetrarrhena* and is characterized by the third and fourth outer glumes being larger than the first and second. The second group, comprising the genera *Phalaris*, *Anthoxanthum* and *Hierochloa* has the third and fourth glumes smaller than the first and second. The two groups are also different in geographical distribution. The first is confined to the southern hemisphere while the second, with the exception of one species of *Anthoxanthum* grows entirely in the northern hemisphere. Both groups have compound starch grains and type 2 leaf anatomy.

Cytologically the tribe is divided into two groups corresponding, as far as present investigations indicate, to the above mentioned taxonomic groups. Avdulov (1) finds that the chromosomes of the first group, represented in his work by *Ehrharta panicea*, are small and in multiples of 12. They therefore resemble the genus *Oryza*. The structure of the starch grains, anatomy of the leaves and geographical distribution are also the same as in the Oryzeae.

The second group is characterized by large chromosomes in multiples of 7 or less. In the genus *Phalaris* the chromosomes are at least three times as large as those of *Ehrharta panicea* and the basic number is 7. *Phalaris canariensis* formed an apparent exception since both Avdulov (1) and Church (10)

reported 12 as its diploid number. Recently, however, Nakajima (35) has established its haploid number as 14 and its diploid number as 28. The only species of this genus examined in the present investigation was *P. arundinacea* (Fig. 16). The diploid number of 28 coincides with that of Avdulov's material.

Anthoxanthum also has large chromosomes. Marchal (1920) (Avdulov (1)) counted 8 chromosomes in the meiotic first metaphase of *A. odoratum*, but Avdulov (1) reported no deviation from 20 chromosomes in somatic tissue; the material examined in the present study also had 20 chromosomes (Fig. 15). *A. aristatum* has 10 chromosomes and therefore the basic number for the genus is probably 5. Two interesting features were observed in the material examined. In one cell it could be seen very clearly that the ends of three of the chromosomes (a, b, and c, Fig. 15) were four-partite instead of bi-partite as is commonly the case. The other feature was the presence, in all the cells examined, of two pairs of large "fragments" and one pair of small, almost spherical, ones (shown in outline in Fig. 15). These "fragments" do not stain as deeply as the rest of the chromosomes, they always lie in definite pairs as shown, and no attachment constriction is visible. They appear to be a constant component of the nucleus, however, as they were found in more than the one root-tip. It seems, therefore, that they may be analogous either to the "chromosomal bodies" reported by Avdulov (2) in *Zea mays* or the "additional chromosomes" reported by Avdulov and Titova (4) in *Paspalum stoloniferum*.

Thus we have two basic numbers in this second group; 7 for the genera *Phalaris* and *Hierochloa* and 5 for the genus *Anthoxanthum*. Avdulov (1) thinks that this difference between the basic numbers may be due to an evolutionary deviation of *Anthoxanthum* away from the more primitive number 7, characteristic of the other members of the group and, indeed, of the majority of the Pooideae.

There seems to be some relationship between the second group and the Aveneae. In *Anthoxanthum* the lower lemmas sometimes bear male flowers and are awned from the back as in the Aveneae. In *Hierochloa* the lower lemmas, or at least one of them, always bear male flowers. The chromosomes of the two groups are also very similar, both in basic number and in morphology. There is also a marked correspondence in their geographical distribution.

On the other hand, the first group of the Phalarideae, of which *Ehrharta panicea* is so far the only representative to be examined cytologically, bears a close similarity to the Oryzeae.

Prat (41) examined the epidermis of *Phalaris paradoxa*, *Phalaris minor*, *Phalaris arundinacea* and *Anthoxanthum odoratum* and considers them to have well marked affinities with the Oryzeae. In the face of this evident disagreement between the two methods of classification it is advisable that further and more detailed study be made, both of their karyology and "dermatology" (Prat), before any definite placing be given to either

of the groups of genera. At present, however, the cytological data seem more conclusive, at least in so far as the second group is concerned. It appears to be fairly certain therefore, that this second group and the Aveneae are quite closely related. Avdulov (1) considers that this resemblance may be taken to represent a side-line development by the genera *Phalaris*, *Anthoxanthum* and *Hierochloa* that has not become differentiated to any great extent.

Tribe Oryzeae

According to Bews (6) "the Oryzeae, or Rice tribe, are particularly interesting for many reasons. They are an isolated group, without any very obviously close connections; in some ways they are rather primitive, as, for instance, in the prevalence of six stamens among them; in other respects they are advanced, the spikelets being reduced to the one-flowered condition, and the glumes often being entirely suppressed or replaced by two minute empty lemmas, or rarely both glumes and empty lemmas may be present as in *Oryza* itself. One whole section (which Hitchcock elevates to the rank of a distinct tribe, the Zizaneae) has the spikelets unisexual and the plants monoecious. In some respects the Oryzeae suggest the Paniceae, particularly in the suppression of the glumes, and Hackel placed them in the Panicoideae. But they differ from the rest of the Panicoideae in having the spikelets laterally compressed, and they are now always included in the Pooideae.

Not only are they somewhat isolated floristically, but they are also very distinct ecologically, for they have responded to a different environment from the majority of the grasses. With the exception of *Lygeum spartum*, all the genera have become adapted to hygrophilous or purely aquatic modes of life. The aquatic forms are the most advanced."

It is evident from morphological and anatomical studies, that the Oryzeae as a tribe do not belong to the Panicoideae. The Oryzeae have clumped starch grains and type 2 leaf anatomy, whereas the Panicoideae have simple starch grains and type 1 leaf anatomy. There is a variation in epidermis within the tribe, however. Grob (1896) (Avdulov (1)) found that the genera *Oryza*, *Zizania* and *Lygeum* possessed the two-celled hair characteristic of the Panicoideae and that the genera *Pharus*, *Luziola*, *Zizaniopsis* and *Hygroryza* lacked them. Prat (41) prefers to group the Oryzeae with the Panicoideae, but his investigations were confined to the genera *Oryza* and *Lygeum*, both of which belong to Grob's Panicoid group.

Similarly, cytological study has been made of only one species, *Oryza sativa* (1). In this case, however, the evidence is against classifying the genus with either of the existing subtribes. Its chromosomes are small like those of the Panicoideae but its basic number is 12. It resembles a number of genera scattered throughout the Pooideae in this respect and, while evidence based on only one genus is naturally rather inconclusive, yet Avdulov (1) prefers to create a special section of the subtribe, Poateae (Hitchcock) Avdulov, for those forms with small chromosomes in multiples of 12.

Subfamily Panicoideae

Bews' (6) description of this subfamily is as follows: "Except in unisexual and neuter spikelets, the spikelets have one terminal perfect floret, with a male floret or empty lemma below it. One glume, or rarely both glumes, may be wanting. The rachilla is not continued above the upper floret. The spikelets fall entire from their pedicels, singly or in groups, or together with the joints of the rachis. The spikelets are usually more or less compressed.

The Panicoid series seems to have had a distinct origin from the Pooideae but may connect somewhat remotely with ancestral forms which resemble the Bamboos. It is obvious that the whole of the Panicoideae are rather highly developed in so far as reduction in the number of flowers in the spikelet is concerned. But, as we have seen, this reduction occurs in primitive tribes of the Pooideae as well. Though a very important evolutionary trend, it is, after all, only one among many. It has taken place within all the separate circles of affinity." Bews (6) considers that there are really only two main tribes of the Panicoideae, for the Maydeae are little more than a subtribe of the Andropogoneae and the Melinideae an unimportant transitional tribe which might be included in the Paniceae.

Tribe Melinideae

This tribe is of little importance. It is rather a heterogeneous collection of genera in which the spikelets have the two heteromorphous florets, the upper perfect, the lower male or barren, characteristic of the whole Panicoid series. The glumes and lemmas are both membranaceous, the latter not harder than the glumes, as in the Paniceae, and the former not much harder either, or at least not closing round the spikelet as in the Andropogoneae. Bews (6) says that Stapf includes the genera *Rhynchelytrum*, *Tricholaena* and *Melinis* in the Paniceae, and *Beckera* in the Oryzeae. Unfortunately, only one of these genera has been examined cytologically.

Avdulov (1) examined the genera *Arundinella*, *Melinis* and *Phaenosperma*. Hackel (16) maintained that *Arundinella* was the genus from which the tribe Andropogoneae originated. Avdulov (1) believes their morphological similarity to be confirmed cytologically through *A. anomala* and *Miscanthus japonicus*. According to his study they have the basic number 9. Two species of *Miscanthus* examined in the present investigation were found to have the basic number 7 and 8 respectively. This may not signify lack of correctness in Hackel's (16) surmise however, since, as has already been noted, it is possible for such a change to occur without producing any great effect on the expression of the phenotype. Avdulov doubts that all the Andropogoneae were derived from the genus *Arundinella*, but rather considers it more probable that *Arundinella* and *Miscanthus* form a connecting link between the Andropogoneae and the Melinideae.

Hackel (16) believed the genus *Melinis* to have sprung from the Paniceae and, as mentioned, Stapf includes it in that tribe. Avdulov (1) examined *M. minutiflora* and found its chromosome number to be 36; this was confirmed (Fig. 17). Since the basic number of most of the Paniceae is 9, and

since most of them have small chromosomes, as has *M. minutiflora*, Avdulov considers it quite logical to regard the genus as a member of the Paniceae.

The third genus which Avdulov investigated was the monotypic *Phaenosperma*, represented by *P. globosa*. It is cytologically very different from the other members of the tribe in that it has the basic number 12. This change in basic number is accompanied by parallel morphological differences which, along with the size of its chromosomes, place it in close relationship with *Oryza*, *Ehrharta* and the group within the Agrostideae represented by *Stipa* and *Oryzopsis*. Hackel (16), Bews (6), and other systematists seem to have erred, therefore, in including this genus in the Panicoideae.

Tribe Paniceae

The Paniceae are less advanced than the Andropogoneae floristically, and this is reflected very clearly in their distribution and ecological behavior. According to Bews: "They are grasses of the great tropical and subtropical regions of the world, where they are abundant. They do not enter very much into the composition of subtropical mesophytic grassland, a type of vegetation which, on the other hand, is completely dominated by the Andropogoneae." It is true that the Paniceae have produced some rather highly evolved ecological types, but the large majority belong to the primitive hygrophilous situations of the tropics. The more advanced types occupy drier situations in the warm regions, and a few, *e.g.* a species of *Paspalum* in South America, occur in grasslands, mixed with the Andropogoneae.

The Paniceae form a very large tribe having, according to Bews (6), about 60 genera and nearly 1400 species. Yet, as is to be expected from the uniformity of their habitat, many of the genera are very closely related, and the tribe as a whole forms one of the most natural groups within the family. Bews (6) follows Stapf in dividing up some of Hackel's (16) genera, chief among which is *Panicum*. Bews' nomenclature is followed in the present discussion of the tribe.

Hackel's section *Digitaria* of the genus *Panicum* becomes Bews' genus *Digitaria*. Avdulov (1) examined two forms of the species *Digitaria sanguinalis* Scop.; *viz.*, *f. australis* Mez. and subsp. *horisontalis* Mez. In both he found 36 chromosomes. *D. exilis* (Fig. 18) was found in the present study to have 54 chromosomes.

The section *Echinochloa* is also raised to the rank of a genus. According to Kihara *et al.* (25), *Panicum crusgalli* L. var. *frumentaceum* Hook. = *Echinochloa crusgalli* Beauv. = *Echinochloa frumentacea* (Roxb.) Link. Hitchcock (20) gives *Echinochloa frumentacea* Link. as synonymous with *Echinochloa crusgalli edulis* Hitchc. Avdulov (1) reports 54 chromosomes in *Panicum crusgalli* L. var. *frumentaceum* (Roxb.) Frimen. Church (11) gave 21 as the haploid number for *Panicum crusgalli* L. var. *frumentaceum* Hook. *Echinochloa frumentacea* (Roxb.) Link. (Fig. 19) examined in the present study had 36 chromosomes. The basic number is the same as in Avdulov's material, and it appears that Church (11) may have been mistaken in his observations.

The section *Brachiaria* becomes Bews' (6) genus of the same name. The genus is based on *Panicum eruciformis* Sibth. (Hitchcock and Chase (21)). Hitchcock (19) gives this species as synonymous with *B. erucaeformis* (J. E. Smith) Griseb. Avdulov (1) reported 18 chromosomes in it. *B. mutica* Stapf has 36 chromosomes (Fig. 21).

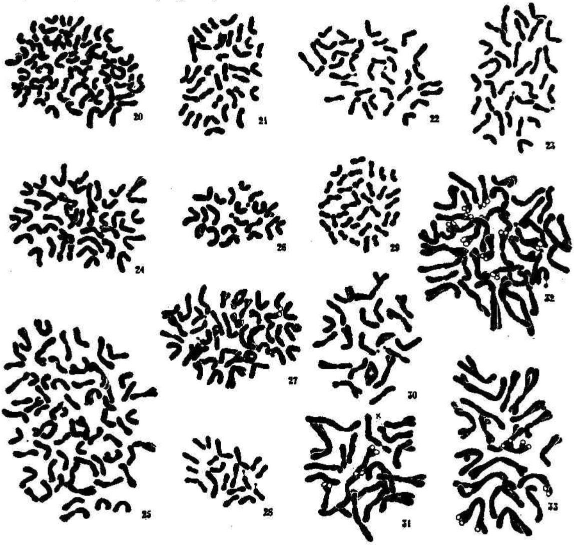

FIG. 20. *Oplismenus burmanii (Retz.) Beauv.*, $2n = 72$. FIG. 21. *Brachiaria mutica Stapf.*, $2n = 36$. FIG. 22. *Pennisetum clandestinum Hochst.*, $2n = 36$. FIG. 23. *Cenchrus tribuloides L.*, $2n = 34$. FIG. 24. *Miscanthus sinensis Anderss.*, $2n = 42$. FIG. 25. *Miscanthus saccharifera Benth.*, $2n = 64$. FIG. 26. *Andropogon eliottii Chapm.*, $2n = 20$. FIG. 27. *Andropogon scoparius Michx.*, $2n = 40$. FIG. 28. *Apluda mutica L.*, $2n = 20$. FIG. 29. *Tripsacum dactyloides L.*, $2n = 36$. FIG. 30. *Coix lachryma-jobi L.*, $2n = 20$. FIG. 31. *Euchlaena mexicana Schrad.*, $2n = 20$. FIG. 32. *Euchlaena perennis Hitch.*, $2n = 40$. FIG. 33. *Zea mays L. subsp. indentata*, $2n = 20$.

Hitchcock (20) makes the section *Ptychophyllum* of the genus *Panicum* a section of the genus *Chaetochloa* Scribn. This latter genus is synonymous with *Setaria* Beauv. Hackel's (16) *P. plicatum* Lam., in which Avdulov counted 36 chromosomes, falls in this group. Of the other species of *Setaria* which Avdulov examined, two have 18 chromosomes and two 36, making a polyploid series with 9 as the basic number.

The remaining section which Avdulov examined, Hackel's *Eupanicum*, Bews retains in his genus *Panicum*. All species investigated have multiples of 9 chromosomes.

Throughout the whole of Hackel's genus *Panicum* there is, then, no divergence from the basic number 9, with one very doubtful exception. All the species are so similar in their chromosome morphology that it is impossible as yet to subdivide them on that score. A very careful comparison of their karyotypes may bring out definite generic differences, but until then their classification must be based on other than cytological grounds.

Avdulov found a characteristic case of polyploidy in the genus *Pennisetum*. Of the species so far investigated, one is a diploid ($2n=18$), one is a triploid ($2n=27$), two are tetraploids ($2n=36$), two are pentaploids ($2n=45$), and two are hexaploids ($2n=54$). To this list has been added the species *P. clandestinum* (Fig. 22) with 36 chromosomes, a tetraploid.

The genus *Pennicillaria* Willd. is included by Bews and Hackel in the genus *Pennisetum* Pers. Avdulov examined *Pennicillaria spicata* Willd. and found that it had 14 large chromosomes, larger than those of any other member of the tribe. In view of its peculiar karyotype, it seems better to retain it in a separate genus, as does Willdenow (quoted by Chase (9)), rather than to group it with *Pennisetum*.

Of the three species of *Oplismenus* which Avdulov examined, one had 54 chromosomes and the other two had 72. In the present investigation *O. burmanii* (Fig. 20) was found to have 72 chromosomes, giving the basic number 9.

In the genus *Cenchrus*, Avdulov finds three species with the basic number 17 and one with 35. *C. tribuloides* (Fig. 23) which was examined in the present study has 34 chromosomes.. Avdulov believes that the number 34 must have originated from a tetraploid form of a series with the basic number 9 through the loss of one pair. The 70-chromosome form, in his opinion, might have arisen through the backcross of a 34-chromosome form with a 36-chromosome form followed by chromosome doubling.

An interesting case is disclosed in the genus *Paspalum*. All the species so far examined have their chromosomes in multiples of 10. This basic number is characteristic of most of the Andropogoneae. In point of chromosome size there is also a decided similarity between the two groups. This evidence along with the fact already noted, that the genus is one of a few that desert the typical habitat of the Paniceae and is found growing mixed with the Andropogoneae, points to the possibility that it should be included with the latter tribe rather than with the Paniceae.

Tribe Andropogoneae

The Andropogoneae are marked by an extreme specialization in the inflorescence. This specialization marks an advance and applies, with variations, to the whole tribe. "Within the tribe, however, are further degrees of advance and specialization, and the genera fall into two series, the one with the joints and pedicels relatively slender and not approximate or fused together—relatively primitive in this respect—and the other more advanced, with the joints and pedicels more or less stout and approximate or fused

together, forming a receptacle of the sessile spikelet." The first series consists of the subtribes Saccharinae and Andropogoninae, and the second of the subtribes Ischaeminae and Rottboellinae.

The Saccharinae are probably the most primitive and its members include *Saccharum* and its allies *Imperata*, *Miscanthus* and *Erianthus*, as well as *Eulalia*, *Polytrias* and several others. They are all relatively primitive ecologically as well as floristically.

The Andropogoninae is a much larger and more progressive subtribe. It includes only seven genera in Hackel's (16) arrangement, for he grouped a large number into the single genus *Andropogon*. Other authorities, however, gave generic rank to the numerous subgenera of *Andropogon*. Stapf has retained most of them and also established several more, so that the subtribe now contains about thirty genera. Some of these are *Andropogon*, *Cymbopogon*, *Sorghum*, and *Amphilophis*, subdivisions of Hackel's *Andropogon*, as well as *Themeda*, *Arthraxon*, and so on. Bews (6) follows this system.

Under the subtribe Ischaeminae come *Apluda*, *Jardinea* and *Elyonurus*, and under the Rottboellinae the most important is *Rottboellia*.

Avdulov (1) examined representatives of a number of genera and found that in most cases the basic number was 10, but that in *Miscanthus*, *Arthraxon* and *Rottboellia* it was 9. In the present investigation two species of *Miscanthus*, *M. sinensis* (Fig. 24) and *M. saccharifera* (Fig. 25), were examined and found to have 42 and 64 chromosomes respectively. Avdulov (1) examined only *M. japonicus* and found 36 chromosomes. We have here an instance of a change in the basic number within the genus. As was mentioned in the general discussion on the use of the karyotype in systematics, it is possible for such a change to occur without producing any great effect on the expression of the phenotype and, since the three species are closely related, they are most likely derivatives of one another.

Avdulov (1) follows Hackel's (16) system of classifying the genus *Andropogon* and finds all the species investigated to have the basic number 10. The species *A. eliottii* (Fig. 26) and *A. scoparius* (Fig. 27) have 20 and 40 chromosomes respectively. Huskins and Smith (23) reported 40 as the somatic chromosome number of *Sorghum halepense* and 20 for twenty-one other species and varieties of *Sorghum*. They found also a number of tetraploid segments and one octoploid segment in root-tips of diploid plants. *S. dimidiatum* Stapf, *S. versicolor* Anders. and *S. purpurea sericeum* Ascher and Schweinf. have since been found to have only 10 chromosomes (18). The chromosomes are much larger than those of the 20-chromosome group, and of very different shapes. The suggestion is made by Huskins (18), that the 10-chromosome sorghums constitute a closely interrelated group very distinct cytologically from the remainder of the genus.

The similarity between the chromosomes in all the species so far investigated, with the exception of the above small group of sorghums, is so great that it is impossible to subdivide the genus *Andropogon* as constituted by Hackel (16) on purely cytological grounds.

In the subtribe Ischaeminae the only genera which have been examined are *Ischaemum* and *Apluda*. Avdulov (1) doubts Kuwada's (1915) (Avdulov (1)) count of 68 in *I. anthephoroides* and gives 20 as the count in *I. timorense*. For *Apluda mutica* he reports 40 chromosomes. The present material (Fig. 28) had only 20 chromosomes, so that it is probably a diploid and Avdulov's a tetraploid.

The whole tribe, then, is characterized by a remarkable regularity in basic number and size of chromosomes. It thus becomes interesting to speculate on the course taken by evolution in the development of the tribe. Avdulov (1) considers that the genus *Miscanthus*, which he postulates as a link between the Andropogoneae and Melinideae, constitutes the point of origin for the whole tribe. From this point development has proceeded in two directions, towards *Imperata* and towards *Erianthus*, in both cases with an increase in basic number from 9 to 10. Further variation in *Erianthus* follows a course through *Pollinia* to *Ischaemum* and *Andropogon* without a change in basic number. From *Ischaemum* it goes in one direction to *Apluda* and in the other direction, with a decrease in the basic number to 9, to *Rottboellia*. *Andropogon* likewise proceeds to *Themeda* without change and to *Arthraxon* with a drop to 9. In addition there is a decrease in basic number from 9 to 8 to 7 within the genus *Miscanthus*.

Tribe Maydeae

There are only seven genera included in this small tribe. Bews (6) considers it as little more than a subtribe of the Andropogoneae. All the members are monoecious, the male and female flowers being in the same inflorescence in some, such as *Tripsacum* and *Coix*, while in *Euchlaena* and *Zea* the male spikes are terminal in a panicle and the female in the leaf axils. The whole tribe is obviously advanced and specialized to a supreme degree, in many ways the most specialized of all the grasses. Within the tribe the genera *Euchlaena* and *Zea* are the most advanced.

The basic chromosome number for most of the genera has been established as 10. Longley (33) reported 35 as the haploid number for *Tripsacum*. Avdulov (1) examined *T. dactyloides* and maintained that it had not less than 72 and probably as many as 80 chromosomes. The material examined in the present study (Fig. 29) definitely had 36 chromosomes. This rather destroys Avdulov's contention that the basic number is probably 10 as is the case with the rest of the tribe. This fact, together with the extreme difference in chromosome size, fits in well however with the acknowledged morphological isolation of the genus.

Avdulov (1) reported 40 as the chromosome number of *Polytoca macrophylla*. This gave it also the basic number 10.

Kuwada (from Avdulov (1)) gave 10 as the haploid number for *Coix lachryma-jobi*. Taylor (49) agreed with him. Avdulov counted 20 chromosomes in the somatic cells. This was substantiated in the present study (Fig. 30). This genus, as well as *Tripsacum*, occupies an isolated position in the tribe, and here again morphological dissimilarity is reflected in the karyo-

type. Its chromosomes, though larger than those of *Tripsacum*, are on the whole considerably smaller than those of the other genera of Maydeae investigated.

The genus *Euchlaena* is *Zea's* closest relative. Kuwada in 1915 (Avdulov (1)) reported 20 as the chromosome number of *E. mexicana*, and Longley (33) counted 40 in *E. perennis*. The present investigations agree with both of these (Figs. 31 and 32). An interesting observation in *E. mexicana* was the discovery of a heteromorphic pair of chromosomes. These are indicated in the figure by an X. This condition was constant in all the cells examined. The material was grown from seed supplied by Dr. G. W. Beadle, who noted on the seed packet that it "might have been contaminated by maize pollen in comparatively recent generations." Only one plant has so far been examined.

In his survey of chromosome numbers in *Zea mays*, Randolph (43) notes that Kuwada (1911, 1915 and 1919) reported the haploid number as varying from 9 to 12, sugary varieties usually having higher numbers than starchy varieties. Longley (33) examined four varieties and found no deviation from 10, but later (34) he found more than 10 in two starchy and two sugary strains. Fisk in 1925 (Randolph (43)) investigated different strains and "found no clear evidence of numbers other than 20 (diploid) except in the sugary variety Black Mexican." Here he found, as did Kuwada, inconstancy of number both in different individuals and in different cells of the same individual. Kiesselbach and Petersen (24) made a large number of counts. No deviations from 10 as the haploid number were discovered. The suggestion was made that the extreme variation reported by Kuwada was perhaps due to errors of counting. Kuwada (27) re-examined the preparations on which his former accounts had been based and found additional evidence in support of his earlier observations. Recent investigations by Avdulov (2) confirm Kuwada's earlier observations. The exact nature of these supernumerary chromosomes, however, is not yet definitely settled. In the present study a variety of Dent corn, *Zea mays indentata* (Fig. 33), was examined and no deviation from 20 chromosomes was found.

A comparison of the Andropogoneae and Maydeae with regard to basic number and chromosome size shows that while the basic number 10 is typical of both, the chromosomes of the latter are very much larger. *Tripsacum* and *Coix* are exceptions and may be said to form a connecting link, cytologically, between the two tribes. Because of this almost general extreme difference in chromosome size it seems more logical to consider the Maydeae as a distinct tribe rather than merely as a subtribe of the Andropogoneae.

Conclusions

On the basis of his investigations Avdulov (1) divided the grasses into three groups. The first group is characterized by (i) type 1 leaf anatomy, (ii) chromosomes in multiples of 9 or 10, rarely more or less, (iii) elliptical or lanceolate first leaf, growing horizontally, (iv) usually a row of small hairs in

place of the ligule, (v) tendency of the main shoot to tiller, (vi) peculiar shape of the silicated cells and the presence of two-celled hairs on the epidermis of the leaf, (vii) alternation in the leaf of one primary with several or many secondary vascular bundles and (viii) limitation almost entirely to tropical or subtropical regions. The group of genera possessing this complex of characters, which includes the Panicoideae, Chlorideae and a number of genera from other tribes, constitutes, therefore, a very natural group. Avdulov thus defines a new subfamily Sacchariferae (Harz) Avdulov.

The second group differs from the first in having (i) its chromosomes in multiples of 7 or less, rarely 9, together with (ii) a linear first leaf growing vertically, (iii) type 2 of chlorophyll arrangement, (iv) the primary vascular bundles of the leaf alternating with one, two, or, at the most, four secondary ones, (v) two-celled hairs entirely absent, (vi) starch grains compound, except a small group in the Hordeae, together with *Bromus* and *Boissiera*, where the starch grains are still different from those of the *Sacchariferae*, and (vii) limitation to the temperate or cold zones.

The third group, *Oryza*, etc., lacks uniformity in many respects. Avdulov (1) has examined only isolated genera of this group, but believes it to constitute a tropical or subtropical group with small chromosomes in multiples of 12 but with a different type of leaf structure from that of the Sacchariferae.

The second and third groups are placed together in the second subfamily Poateae (Hitchcock) Avdulov, the second as the Festuciformes Avdulov, and the third as the Phragmitiformes (Harz) Avdulov. The group Festuciformes is subdivided into two tribes, the Frumentaceae (Harz) Avdulov, and the Festucaceae (Kunth) Avdulov, the first with simple and the second with compound starch grains.

The present investigation with a few minor exceptions supports Avdulov's conclusions. It is evident that parts of the family have not yet received sufficient attention and that more work must be done on them before any really valid conclusions can be reached. This applies in particular to Avdulov's third group which at present comprises a more or less heterogeneous collection of genera bound together only by rather superficial resemblances. The tribe Bambuseae is also deserving of more attention. Many investigators claim that it forms a subfamily of its own which is probably intermediate in position between the other two subfamilies or from which they have developed. Some smaller groups seem incapable of further development along cytological lines. In such a case all that cytology can do is indicate the existence of a more or less close relationship between its members. An examination of such groups confirms the important fact that was brought out in the introduction, *viz.*, a karyotype cannot be exclusively applied to any particular systematic unit. We are thus brought to a recognition of the fact that cytology can no more be used as the sole basis of classification than can any single form of anatomical or morphological study. It can, however, when properly used and correctly interpreted, form a very powerful tool in the hand of the system-

atist who is willing to correlate its facts with those derived from other branches of study. When two or more unrelated lines of investigation point to the same conclusion, the probability of that conclusion being correct is, naturally, very greatly enhanced.

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A NEW *OPHIOBOLUS* ON EELGRASS[1]

By Irene Mounce[2] and Wm. W. Diehl[3]

Abstract

Ophiobolus halimus Diehl and Mounce n. sp. is reported on *Zostera marina* L. from St. Andrews, N.B. It was collected on rhizomes and fertile shoots, and developed in leaves kept in sea water in the laboratory. The fungus is described and illustrated and it is compared with related species.

Introduction

The almost complete disappearance of eelgrass, *Zostera marina* L., during the seasons 1930-1933 from the western Atlantic coast and the development of a similar condition in Europe have excited considerable interest not only among botanists, but among the public generally. Since the cause of these phenomena has lacked adequate explanation, any organisms found growing on or in the eelgrass plants are of possible significance. It seems desirable, therefore, to record a hitherto unknown fungus on *Zostera* which has been under observation in the field and in culture, and to distinguish it from related species with which it may be confused.

Field Observations and Cultures

In co-operation with the Biological Board of Canada, a preliminary investigation of the conditions affecting eelgrass was begun last autumn near their station at St. Andrews, N.B., by the senior author.

Most of the observations on *Zostera* were made at Tidal Cove and Birch Cove, two closely adjacent inlets of Passamaquoddy Bay. A dam was built across the upper end of Tidal Cove some years ago and in the tidal pools behind it the supply of *Zostera* was fairly abundant, whereas at Birch Cove only a few scattered youug plants remained.

Perithecia of an ascomycetous fungus occurred in great numbers on the lower parts of most of the fertile shoots which remained at Tidal Cove in late August and September. At that time, few spores were present but they were sufficiently abundant that the fungus could be characterized by their size, shape, and hyaline tips.

Mature perithecia have been found in the rhizomes of a few sickly plants at both Birch Cove and Tidal Cove (Figs. 7, 9). In such rhizomes the cells are filled with septate hyphae (Figs. 8, 9).

Perithecia developed, too, on the rhizome of one plant of *Zostera* which had been kept in a jar of sea water in the laboratory at room temperature,

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Contribution from the Dominion of Canada Department of Agriculture, Ottawa, Canada and the United States Department of Agriculture, Washington, D.C., U.S.A.

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The senior author is responsible for all field and culture work with this fungus and the junior author for the taxonomic considerations.

but the greatest numbers of mature perithecia were obtained from leaves (Figs. 1, 3). Tips of apparently healthy leaves were placed in test tubes or flasks, which had been partially filled with sea water and plugged with cotton wool, and kept either at room temperature or partially immersed in a tank of running sea water where the temperature ranged from 13.5°–15° C. At the end of six weeks to two months development was complete.

Perithecia have matured on leaves collected in September and October, and on some which were sent to Ottawa from St. Andrews early in January. Because of the larger amount of this latter material it has been used as the basis for taxonomic consideration.

Taxonomic Relations

This fungus is readily identified as belonging to the genus *Ophiobolus*, but it appears to differ from any described species of that genus as outlined below:

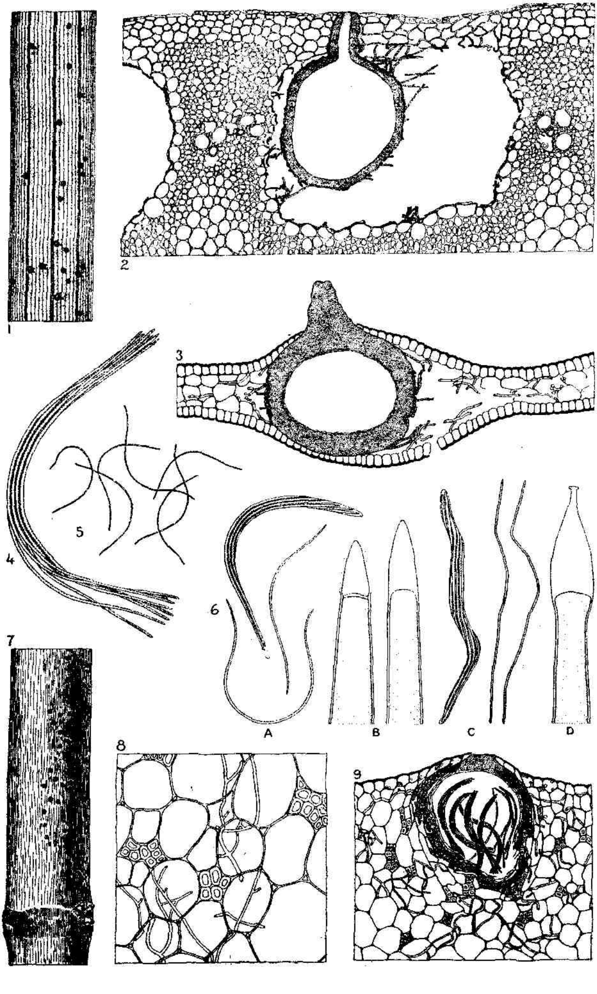
Ophiobolus medusa

A little-known species, *O. medusa* Ell. and Ev., (1, p. 150) which was found in 1884 in New Jersey growing upon sand-buried leaf sheaths of *Spartina* sp.; in reality *S. cynosuroides* (L.) Roth., does bear a certain remarkable resemblance* due to a character which is not noted, however, in the original description. This feature common to *O. medusa* and the fungus on *Zostera* is the hyaline tips or apiculi of the ascospores. No attempt is made here to determine the nature of this apiculus other than to note that it seems not to be an evaculated cell but a solid hyaline cap. In *O. medusa* the tip of this cap or apiculus tends to be inflated and flattened somewhat (Fig. 6*D*), a character that is readily overlooked unless examined with an oil-immersion lens. In the fungus on *Zostera* the ascospore apiculus terminates normally as a rounded cone (Fig. 6*B*).

In *O. medusa* the asci are described as more than 400μ long, being larger than those of the fungus on *Zostera* where the equivalent dimensions are usually 270-300μ. In the microscopic preparation from the type of *O. medusa* the asci and spores are somewhat smaller than in the description owing doubtless to the fact that they are slightly immature. The ascospores of *O. medusa* have a densely staining central cell (Fig. 6*C*) which is less evident in the fungus on *Zostera*, and in the latter there is a spring-like turgidity lacking in *O. medusa* (Figs. 5; 6*A*). It is, of course, always difficult to make fair comparisons of spores from dried specimens with those from fresh material.

The texture of the perithecial walls and of the ostiolar beak of *O. medusa* is heavier and less membranous than in the fungus on *Zostera*. The perithecia of *O. medusa* are surrounded by attached brown hyphae (hairs) which are not evident in the fungus on *Zostera*. In *O. medusa* the ostiolar beak does not protrude (Fig. 2) from the tissues of the host while in the fungus on *Zostera* they are decidedly exsurgent (Fig. 3).

**Dr. John Dearness in a communication to the senior author suggested that if the fungus on Zostera were not a form of O. medusa it was probably a new species.*


1
2
3
4
5
6
7
8
9
A
B
C
D

These features in *O. medusa* have been observed in slides made from the fragmentary type specimen in the Ellis Collection of types of the Farlow Herbarium at Harvard University. Another duplicate type (syntype), that is in the Ellis Herbarium of the New York Botanical Garden, was examined, but no ascospores were found. No other specimen of *O. medusa* has been available thus far although the junior writer has searched for it on buried *Spartina cynosuroides* during the spring of 1934 in the Chesapeake Bay region.

Ophiobolus medusa var. *minor*

A supposedly related fungus, *O. medusae* var. *minor* Ell. and Ev. (2, p. 239) described from *Andropogon muricatus* at St. Martinville, La. in 1889 by A. B. Langlois, as attested by the type specimen in the Langlois Herbarium (A.B.L. 1771), in the Mycological Collections of the U.S. Bureau of Plant Industry, has small asci 110 – 120 × 7–8μ and spores 90 – 110 × 2.5μ, which lack the peculiar appendages referred to above, and is characterized by an erumpent ostiolar beak. A proper disposition of this fungus from Louisiana would be elsewhere than with *O. medusa* and it can have no bearing upon the taxonomy of the fungus on *Zostera*.

Ophiobolus maritimus

A discussion of *Ophiobolus* on *Zostera* cannot ignore the record of *O. maritimus* Sacc. (3, p. 350) described from Europe as growing in the North Sea on *Z. marina*. Although no specimens of this fungus are available for comparison, it also would seem different from the fungus on *Zostera* from New Brunswick, since it is described with much smaller asci, 140–150 × 10–12μ, and 15–18 septate spores which break up into uniseptate segments 15–20 × 2μ in dimensions.

Since this fungus on *Zostera* cannot be identified with either of those with which for various reasons it might have been confused, it is proposed as new:

Ophiobolus halimus Diehl and Mounce, sp. nov.

Perithecia sparse and somewhat equally arranged in groups, intramatrical, sphaeroid, 240–435μ in diameter, with rostrate ostioles emerging amphigenously, sometimes with more than one ostiole to the perithecium; rostra conoid, acute, blackish, up to 278μ in length by 260μ in basal diameter;

EXPLANATION OF FIGURES

FIG. 1. *Ophiobolus halimus. Perithecia in leaf of Zostera marina.* × 6. FIG. 2. *Ophiobolus medusa. Drawing of a freehand section of a perithecium from type specimen, showing the perithecium within an air space of the host and the intramatrical ostiolar neck.* ×103. *Section made by Dr. G. D. Darker and preserved in Amann's solution.* FIG. 3. *O. halimus. Drawing from a freehand section of a perithecium in a leaf of Z. marina. ×103.* FIGS. 4–5. *O. halimus. Spores.* ×273 *and ×103 respectively.* FIG. 6. *A. O. halimus. Ascus and spores. ×182. B. O. halimus. Ascospore tips showing appendage. ×910. C. O. medusa. Ascus and spores. ×182. D. O. medusa. Ascospore tips showing appendage. ×910.* FIG. 7. *O. halimus. Distribution of perithecia on rhizome of Z. marina. ×6.* FIG. 8. *O. halimus. Septate hyphae in cells of rhizome. ×273.* FIG. 9. *O. halimus. Perithecium and spores in a rhizome of Z. marina ×103.*

perithecial walls 9–15μ in thickness, context blackish brown, fleshy pseudoparenchyma of thin-walled cells laterally compressed; asci arcuate-fusoid, slightly attenuate at the base, subsessile, apically somewhat truncate, 270–300 × 12–15μ, eight-spored, ascospores parallel but slightly spirally arranged and filling the ascus, aparaphysate; ascospores filiform, acicular arcuate to spring-like spiral when free, gradually tapering from the thicker centre to the tips, 260–308 × 2–4μ, spores terminated by a peculiar hyaline appendix which reached a length of 3μ and a diameter of 1.3μ.

Parasitic in leaves of *Zostera marina* in culture in 1934, I. Mounce, Herb. Div. Bot. Ottawa 3584, (type); and naturally on rhizomes and bases of fertile shoots of *Z. marina*, St. Andrews, N.B., August-October, 1933, I. Mounce.

Ophiobolus halimus Diehl and Mounce, sp. nov.

Peritheciis sparsis et equaliter gregariis, intra matricem immersis, atris, sphaeroideis, 240–435μ diam. ostiolis rostratis amphigenis emergentibus, rostris ostiolorum conoideis acutis, atris 278μ altis, 260μ latis; muris peritheciorum 9–15μ crassis, contextu atro-fuliginoso, carnoso pseudoparenchymatico, e cellulis subregularibus, lateraliter subcompressis et muris tenuibus praeditis efformato; ascis arcuato-fusoideis, basi obtuse attenuatis, subsessilibus, apice tenuiter truncatis, 270–300 × 12–15μ, octosporis; aparaphysatis; ascosporis parallelis vel paulo subspiraliter in asco dispositis, acicularibus arcuatis, in medio crassiusculis, hyalinis, pseudoseptatis, appendiculis terminalibus hyalinis, 260–308 × 2–4μ.

In foliis *Zosterae marinae* parasitans in cultura I. Mounce 1934 (typus); et naturaliter in sinu maris prope St. Andrews, in Nova Brunsvigia, 10 Septembris 1933. I. Mounce.

Type material of this species has been deposited in the following herbaria: Division of Botany, Ottawa; the Mycological Collection of the United States, Bureau of Plant Industry; the Royal Botanic Gardens, Kew; the New York Botanic Garden; and the Farlow Herbarium of Harvard University.

Acknowledgments

The authors are much indebted to Miss Ruth Macrae for most of the drawings, to the Biological Board of Canada for additional collections of *Zostera marina*, to Dr. G. D. Darker for sectioning and preparing slides from the type of *Ophiobolus medusa* at the Farlow Herbarium, and to Dr. F. J. Seaver for permission to examine the type specimen of the same species at the New York Botanical Garden.

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REVIEWS AND NOTES

NOTE SUR LES ÉMULSIONS DE LA TRIÉTHANOLAMINE AVEC LE KÉROSÈNE ET DES HUILES LUBRIFIANTES MINÉRALES[1]

PAR J. RISI[2] ET H. BERNARD[3]

La triéthanolamine (4, 5) donne avec les acides gras des savons (1, 3, 8), lesquels forment avec diverses substances minérales et végétales des émulsions aqueuses plus ou moins stables. Nous avons eu l'occasion d'étudier des émulsions de la triéthanolamine avec le kérosène et des huiles lubrifiantes minérales, et nous voudrions signaler quelques propriétés autres que celles déjà données dans la littérature (7).

Emulsions de kérosène

La stabilité et l'homogénéité des émulsions dépendent beaucoup de la méthode de préparation, ainsi que des variations même très minimes de la viscosité, de l'acidité et du poids spécifique des produits de départ. Les meilleurs résultats au point de vue de la stabilité ont été obtenus par émulsification de 87 g. de kérosène, 9 g. d'acide oléique, 4 g. de triéthanolamine, 50 g. d'eau distillée, d'après les méthodes connues (1, 3, 8). Cette émulsion concentrée à 66% d'huile forme une crème visqueuse, blanche, stable pendant plusieurs mois et miscible à l'eau. Elle possède les propriétés suivantes:—poids spécifique; 0.878 à 15.56° C.: point de liquéfaction; —1° C.: point d'inflammabilité; 97° C.: point de combustion; 95° C. après avoir brisé l'émulsion par ébullition prolongée: essai de corrosion; négatif pour le cuivre et pour les tissus végétaux: propriétés détersives; aussi prononcées que celles d'une solution de savon neutre ordinaire.

Emulsions d'huile minérale

Dans le but d'obtenir une huile lubrifiante soluble, nous avons émulsifié le savon oléique de la triéthanolamine avec une huile minérale raffinée de P.E. 300–327° C. ne contenant aucune addition d'huile animale ou végétale. Les meilleurs résultats ont été obtenus avec 89 g. d'huile, 3.9 g. de triéthanolamine, 7.1 g. d'acide oléique et 50 g. d'eau distillée. Cette émulsion concentrée, à 66% d'huile, est une crème assez visqueuse, presque blanche, parfaitement neutre et qui s'émulsifie très facilement en ajoutant graduellement de l'eau avec agitation modérée. Ses propriétées sont les suivantes:—poids spécifique; 0.763 à 15.56° C.: point de liquéfaction; − 4° C.: point

[1] *Manuscrit reçu le 4 mai, 1934.*
Contribution de l'Ecole Supérieure de Chimie, Université Laval, Québec, Canada. Note basée sur une thèse présentée par Hervé Bernard pour l'obtention du degré de M.Sc. de l'Université Laval.

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[3] *Etudiant gradué, Université Laval.*

d'inflammabilité; 105° C.: point de combustion; 114° C.: essai de corrosion; négatif: stabilité; la couche supérieure se solidifie après 24 heures, lorsque l'émulsion est exposée à l'air, grâce à une inversion par l'anhydride carbonique (2, 6).

Le tableau I donne les coefficients de friction, μ, des émulsions à 60, 40 et 30% d'huile déterminés dans l'appareil de Thurston. Les coefficients de friction de l'huile à moteur "Marvelube Twenty" de viscosité 315 sec. à 100° F. sont donnés pour comparaison.

TABLEAU I

COEFFICIENTS DE FRICTION DE QUELQUES ÉMULSIONS À BASE DE TRIÉTHANOLAMINE ET D'UNE HUILE À MOTEUR

Minutes	Emulsions			"Marvelube Twenty" μ	*Minutes*	Emulsions			"Marvelube Twenty," μ
	66% d'huile, μ	*40%* d'huile, μ	*30%* d'huile, μ			*66%* d'huile, μ	*40%* d'huile, μ	*30%* d'huile, μ	
0	0.067	0.060	0.060	0.060	7	0.026	0.058	0.053	0.060
1	0.067	0.011	0.017	0.035	8	0.026	0.043	0.059	0.060
2	0.067	0.011	0.017	0.035	9	0.026	0.043	0.066	0.060
3	0.038	0.011	0.023	0.035	10	0.026	0.043	0.071	0.060
4	0.033	0.040	0.047	0.035	11	0.026	0.058	0.071	0.060
5	0.029	0.058	0.047	0.035	12	0.026	0.070	0.071	0.060
6	0.026	0.058	0.047	0.060	13	0.026	0.085	0.071	0.060
					14	0.026		0.085	0.076

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ON THE THERMAL CONDUCTIVITY OF DIATOMITE AT MODERATELY HIGH TEMPERATURES[1]

By C. D. Niven[2]

Abstract

An examination was made of various kinds of diatomite brick, in order to ascertain how the thermal conductivity of diatomite found at Quesnel in British Columbia compared with the conductivities of some other diatomites. A brief description is given of the difficulties which attend any endeavor to measure thermal conductivity with accuracy above 1500° F. and which necessitate the limiting of the investigation to temperatures between atmospheric temperature and 1500° F. Of the samples investigated the Quesnel brick was found to be the best thermal insulator with the exception of a brick consisting of natural Californian diatomite.

Introduction

As an insulator at high temperature there are few substances which can surpass diatomite. This material is found in deposits in various parts of the world, notably in California, in the Island of Moler off the north coast of Denmark, and in Canada. It "consists of minute fossil envelopes of diatoms, a family of unicellular plants," and after it is calcined, it is about 90% silica. The deposits often occur along with clay. When this is so, the clay is sometimes used to bind the diatomaceous earth together in the manufacture of insulating bricks. The insulating qualities of these bricks are of greater importance than their structural strength, as they are used for the inside of furnace walls—the outer faces of which can be made of fire clay brick on the one side and of ordinary brick on the other.

In central British Columbia valuable deposits of this diatomaceous material occur at Quesnel, and the Department of Mines in Canada have been anxious to ascertain whether the British Columbian product would compare favorably with other diatomites as a thermal insulator. On account of the fact that diatomite can be used for insulation at very high temperatures, it was considered desirable to carry out the investigation at the highest mean temperature possible; but when the matter was looked into, the difficulties of

[1] *Manuscript received June 6, 1934.*
Contribution from the Division of Physics and Engineering, National Research Laboratories, Ottawa, Canada.
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accurately measuring thermal conductivity at very high temperatures appeared to be very considerable if not entirely insurmountable. At any rate no standard method had been developed for such work.

Three principles are available for estimating the transmission of heat energy: first, the measurement of heat supplied; second, the measurement of the heat which comes through the insulator and is absorbed on the cold side; third, the measurement of heat flow across any section parallel to the hot or cold plate. Each of these methods has its disadvantages.

The first principle, which in point of fact was the one employed by the writer, cannot be used for hot plate temperatures above 1600° F. This results from the fact that no material which is a good electrical insulator at these temperatures has yet been found and therefore no material is available, upon which to wind the heater wire supplying the thermal energy, without involving an electrical leak whereby the accuracy of the results would be vitiated.

The second principle known as the calorimeter principle depends upon the flow of water at constant speed through the cold plates. The rise in temperature of the water gives a measure of the heat transmitted. This method, however, practically fixes the cold plate temperature at about 100° F. so that to reach a mean temperature of 1500° F. the hot plate temperature would have to be in the neighborhood of 2900° F. This principle, therefore, does not permit of any higher mean temperatures than the first one; it works satisfactorily and is used at the National Physical Laboratory in England.

The third principle is still in the experimental stages and need not be discussed here. If one can get a satisfactory flow meter, this method has the overwhelming advantage over the other two, that neither the temperature of the hot, nor the temperature of the cold plate, need be limited to narrow ranges since neither plate is used for the measuring of the energy transmitted.

Apparatus

A design of apparatus was employed, very similar to that described by Hartmann, Westmont and Weinland (1). It will be necessary therefore to refer only to the main features of the design.

This type of apparatus differs from the ordinary hot plate apparatus in that the "cold" plates also are heated, and so comparatively high temperatures can be obtained on the cold side of the sample. The cold plate consists essentially of four parts, namely, a water-cooled plate, a piece of sil-o-cel insulation, an electrically heated alundum plate, and a nichrome disk—the last mentioned being employed to give equal distribution of heat over the cold face of the test sample.

The hot plate itself and the two heated plates used in the combinations which formed the cold sides were constructed from double-spiral-grooved alundum plates. The heater wire which rested in this double-spiral groove was a No. 22 nichrome wire. Nichrome is less expensive than platinum and must therefore be used, but great trouble was caused by the wire oxidizing

after long periods of heating at the high temperatures necessary for the work. The hot plate was equipped, of course, with a guard-ring, and platinum-platinum-rhodium thermocouples cemented into the surface of the plate determined when the guard-ring was at the same temperature as the central portion of the plate.

The energy supplied per square foot of plate surface was estimated by measuring the current flowing through the nichrome wire and the difference of potential at two points on the wire; these two points were situated roughly at the points at which the nichrome heater wire crossed the circumference of a circle of a radius of two inches, the centre of which was at the centre of the plate. The actual area of this circle was measured with a planimeter on a carbon rubbing, taken after the spiral had been wound and the potential leads welded on. The energy supplied per square foot of surface was thus the product of a voltage reading, an amperage reading and a constant, obtained once and for all for a particular plate. Mention might be made again of the necessity of using a good electrical insulator for the substance of which the heater plate is composed; the very method of winding, namely by means of a double spiral, implies that the electrical leak—which of course depends on the difference of potential—would vary over a plate which was slightly conducting. It would be greatest at the edge and smallest at the centre.

Experimental Procedure

The materials for testing were provided in the form of bricks. These samples had to be cut to form a slab one inch thick and eight inches in diameter. In order that no join might come in the central four inches of the slab, the following method of cutting was adopted. The brick was cut in two, parallel to the face of the largest area; then one of the two halves so obtained was cut diagonally. These two triangular pieces were placed one on either side of the other, or uncut, half of the brick, and the eight-inch circular slab was cut out. The thickness of a slab thus prepared was measured in a micrometer gauge, and then two platinum-platinum-rhodium thermocouples were fastened, one on either side, with de Khotinsky wax. The couples were sunk into the faces of the sample, so that the junction was flush with the surface. Half of the sum of the thicknesses of the two couples was subtracted from the micrometer reading and the resulting value was taken as the thickness measurement. In placing the samples in the apparatus a thin sheet of asbestos cardboard was placed between the sample and the nichrome disk of the cold plate combination, in order to insulate electrically the thermocouples from that disk.

As a rule it took from two to four days to find the values corresponding to a steady flow of heat. The steady state was taken at mean temperatures in the neighborhood of 500°, 1000° and 1500° F. In this way, the variation of thermal conductivity with temperature was ascertained.

Results and Conclusions

The results obtained are given in Table I. These are shown again in Fig. 1 where thermal conductivity has been plotted against temperature. The

TABLE I

Description of sample	Reference number on Fig. 1	Density (lb./cu. ft.)	Temperature (° F.)	Thermal conductivity (B.T.U./hr./sq. ft./° F./in.)	Remarks
Californian diatomite: clay bond; calcined	1 (a)	40.1	441 997 1378	1.54 1.80 1.91	
Californian diatomite: clay bond; calcined	1 (b)	38.6	196 471 673 892 1193 1386 1557	1.53 1.63 1.74 1.84 1.98 2.05 2.14	
Californian diatomite: cut out of the deposit; no bond; not calcined	2	29.7	536 887 1229 1437	0.556 0.666 0.677 0.666	This brick was not calcined and showed marked change in color at higher temperatures; a slight contraction was also noted
Californian diatomite: a brick supposed to be specially designed for high temperatures	3	38.4	462 988 1217 1412	1.33 1.54 1.57 1.59	This brick was supposed to stand very much higher temperatures than the maximum at which it was tested but it showed a slight change of color as a result of testing at 1412° F.
Moler Island diatomite: clay bond	4	29.1	474 1020 1406	0.906 1.18 1.41	Deep holes were visible on the surface of this sample; it is understood that something is put in, in the manufacture of this brick which is subsequently burnt out
Moler Island diatomite: clay bond	5	49.3	432 1056 1517	1.31 1.53 1.72	
(Uncertain origin; probably mixture of Californian and Algerian diatomite)	6	32.0	440 965 1437	1.04 1.29 1.59	Samples 6 and 7 are probably a mixture of Californian and Algerian diatomite; before calcining it is understood they have a percentage of cork granules which are subsequently burnt out. No. 6 is a reddish colored brick. No. 7 is white
(Uncertain origin; probably a mixture of Californian and Algerian diatomite with a different bond from No. 6)	7	37.8	455 1080 1517	1.22 1.63 1.92	
British Columbian diatomite from Quesnel: clay bond; calcined	8	30.7	490 1092 1499	0.801 1.02 1.17	This brick is rather easily broken and is softer than the other bricks

relation between thermal conductivity and temperature for each sample appeared in general to be expressed by a straight line. The accuracy of the work did not justify the drawing of curves through points which did not strictly obey the linear relation. By extrapolation one may form an estimate of the insulating value of the materials at higher temperatures than those at which the experimental work was undertaken, but such a procedure implies that no change takes place in the physical state of the sample in question, in the region of temperature over which the extrapolation is made.

The work definitely shows that in the range of temperature between 400 and 1500° F. the conductivity of the Quesnel sample is less than that of any of the other samples except the natural Californian diatomite brick which is cut out of the solid deposit and does not contain what is known in ceramics as a "bond."

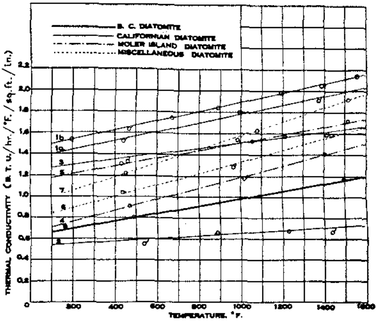

Fig. 1

Acknowledgments

The author takes pleasure in acknowledging many courtesies shown by the Johns-Manville Laboratories, and in particular by Mr. C. E. Weinland, who gave much valuable advice in connection with the construction of the testing apparatus.

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LONGITUDINAL WAVES IN CYLINDERS OF LIQUID, IN HOLLOW TUBES AND IN SOLID RODS[1]

BY GEO. S. FIELD[2]

Abstract

A consideration of recently published results on the transmission of longitudinal waves in cylinders of liquid, in hollow tubes and in solid rods shows that the first two cases give velocity-frequency curves which are quite similar to each other but different from that obtained with the third case. The reason for these results is discussed and is shown to be most probably the relative importance of shear forces in each case. The effect of viscosity on the phase velocity of longitudinal waves is considered, and it is shown that the classical method of introducing viscosity leads to erroneous results.

Introduction

As the result of an investigation on the transmission of sound in liquids contained in cylindrical tubes, it was shown (5) that a discontinuity in the relation between velocity and frequency occurs when the frequency of the longitudinal vibration approaches that of the fundamental radial vibration. The plotted results have the appearance of Curve 1, Fig. 1.

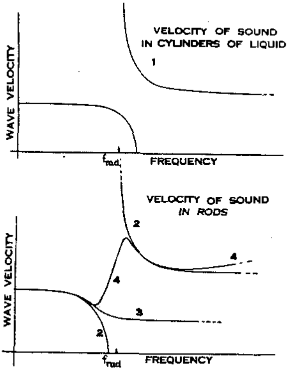

FIG. 1. *Variation of phase velocity with frequency for sound in liquids and in rods. f_{rad} is the frequency of radial resonance.*

Recently a paper was published (6) on the longitudinal oscillations of solid rods and tubes, and a curve was given showing velocity plotted against frequency for a nickel tube. This curve resembles Curve 2, Fig. 1, and can be obtained theoretically, as the authors show, by a consideration of the simple theory of coupled oscillations. The results for solid rods, however, could not be explained so simply.

The vibrations in solid rods have been considered theoretically by Ruedy (12) and Field (4), and a paper containing some excellent experimental work was recently published by Röhrich (11). The variation of wave velocity with frequency is essentially as given by Curve 3, Fig. 1.

It seems of interest to determine why the occurrence of radial vibrations does not cause an abrupt change in the phase velocity of longitudinal waves in a solid rod, when it does do so in the other two cases. In what follows a reason for this behavior is advanced.

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The Importance of Shear Forces

If, in the derivation of the velocity equation for solid rods, the forces due to shear be omitted, that is, it be assumed that the *net* shear strain is zero, the resulting equation is,

$$c_1 = \alpha\sqrt{1-\left(\frac{\sigma}{1-\sigma}\right)^2\frac{f_r^2}{f_r^2-f^2}} \quad *$$

where, c_1 = phase velocity;

$\alpha = c_o\sqrt{\frac{1-\sigma}{(1+\sigma)(1-2\sigma)}}$ = velocity of compressional wave in an infinite solid;

$c_o = \sqrt{E/\rho}$ = velocity of sound in a long, thin rod;

E = Young's modulus; ρ = density; σ = Poisson's ratio;

f = frequency; $f_r = \frac{x\alpha}{2\pi a}$; a = radius of rod;

x = solution of equation

$$x\frac{J_1(x)}{J_o(x)} = \left(\frac{1-\sigma}{1-2\sigma}\right)\frac{4\pi^2a^2f^2}{\alpha^2} \ .$$

Upon plotting this equation, Curve 2, Fig. 1, is obtained, in which again the discontinuity in phase velocity appears, though it did not appear when shear forces were included. It seems reasonable to conclude, therefore, that it is these shear forces which tend to keep the phase velocity from becoming either very small or very large.

It is of interest to derive the velocity equation again†, this time with factors introduced to take care of viscosity. If such be done, Curve 4 is obtained. It will be observed that viscous forces, as well as shear forces, tend to eliminate high and low values of phase velocity, though the actual variation of velocity with frequency is different in the two cases.

Some idea of the way shear forces affect the longitudinal waves in a rod may be obtained if the changes in the shape of the rod as it vibrates are considered. Axial vibrations (on account of the lateral strain accompanying the longitudinal strain) produce radial waves, and hence cause periodic changes in the radius along the rod. Fig. 2 shows (very much exaggerated) how the rod responds to such waves travelling along it. It will be observed that shear waves are forced along the rod at the velocity of the longitudinal waves. If shear waves were oscillating freely along the rod, however, their velocity would be $c_o\sqrt{\frac{1}{2(1+\sigma)}}$, which, for duralumin, is a velocity only about 60% of that of a longitudinal wave in a long, thin rod. The longitudinal velocity, however, drops fairly rapidly as the frequency of the fundamental radial vibration is reached, until it approaches the velocity of shear waves as given above. At this value the shear waves accompanying

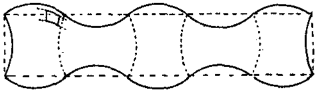

FIG. 2. *Exaggerated view of vibrating rod.*

* *See Equation (13), page 259.* † *See Equation (16), page 260.*

the longitudinal waves are in resonance with their natural frequency, and hence tend to maintain the velocity of the whole system of waves, *i.e.*, longitudinal and shear waves, at this value. Apparently this resonance effect is sufficient to prevent the velocity of the axial vibration from dropping much lower, and it continues near this value to higher and higher frequencies. (See Curve 3).

For liquids contained in tubes, of course, shear forces play no part. Hence, as the frequency is varied through the frequency of radial resonance, the phase velocity tends to become very low and then very high, limited only by viscosity.

In the case of thin tubes, also, shear forces are relatively unimportant. As radial vibrations occur, distortion of the walls into some such shape as Fig. 2 is accompanied by extension and bending, rather than by shearing. Therefore, in the absence of effective shear forces, a discontinuity in the velocity-frequency curve appears.

Reference to Equation (1) shows that at frequencies greater than that of the absorption band the velocity of compressional waves in an infinite solid is approached. Experiments (2, 3) have shown that the longitudinal velocity of sound in thin disks has nearly this value, so it seems reasonable to conclude that in such disks shear forces are relatively small.

I. Derivation of Velocity Equation with Shear Forces Omitted

Axial Strains in a Unit Cube

Elastic theory (1, p. 110, Equation (6)), gives the following equations,

$$f_1(1+\sigma)(1-2\sigma) = a(1-\sigma) + (b+c)\sigma$$
$$f_2(1+\sigma)(1-2\sigma) = b(1-\sigma) + (c+a)\sigma$$
$$f_3(1+\sigma)(1-2\sigma) = c(1-\sigma) + (a+b)\sigma \qquad (1)$$

where f_1, f_2 and f_3 are the individual axial strains caused by the axial forces, p_1, p_2 and p_3, respectively, acting mutually at right angles to each other, and a, b and c are the *total* axial strains in the respective three directions. σ is Poisson's ratio.

Stresses and Strains in an Element of a Cylindrical Rod

Using the cylindrical co-ordinates, r, θ and z, consider an element with sides $rd\theta$, dr and dz.

Let z move to $z+\xi$. Then $z+dz$ moves to $z+dz+\xi+\frac{\partial\xi}{\partial z}\,.\,dz$. The elongation of the element is $\frac{\partial\xi}{\partial z}\,.\,dz$ and the strain is $\frac{\partial\xi}{\partial z}$.

Similarly, when r moves to $r+\zeta$, the strain is $\frac{\partial\zeta}{\partial r}$, and when θ moves to $\theta+\eta$, the strain is $\frac{\partial\eta}{\partial\theta}$.

In this last case, however, there is an additional strain caused by the radial motion along r. An element $rd\theta$ wide is increased to a width $(r+\zeta)d\theta$ owing

to the radial motion through a distance ζ. The strain thus caused is $\frac{\zeta}{r}$.

Hence the total strain in the direction determined by $rd\theta$ is, $\frac{\partial\eta}{\partial\theta}+\frac{\zeta}{r}$.

Finally, we can write,

Total strain along $z=\frac{\partial\xi}{\partial z}=a$,

Total strain along $r=\frac{\partial\zeta}{\partial r}=b$,

Total strain along $rd\theta=\frac{\partial\eta}{\partial\theta}+\frac{\zeta}{r}=c$. (2)

If Young's modulus be denoted by q, $p_1=qf_1$, $p_2=qf_2$ and $p_3=qf_3$.

Substituting for the f's from Equations (1), and using a, b and c as given by Equations (2), the p's may be expressed as follows:

$$p_1=\frac{q}{(1+\sigma)(1-2\sigma)}\left[\frac{\partial\xi}{\partial z}(1-\sigma)+\sigma\left(\frac{\partial\zeta}{\partial r}+\frac{\partial\eta}{\partial\theta}+\frac{\zeta}{r}\right)\right]$$

$$p_2=\frac{q}{(1+\sigma)(1-2\sigma)}\left[\frac{\partial\zeta}{\partial r}(1-\sigma)+\sigma\left(\frac{\partial\xi}{\partial z}+\frac{\partial\eta}{\partial\theta}+\frac{\zeta}{r}\right)\right]$$

$$p_3=\frac{q}{(1+\sigma)(1-2\sigma)}\left[\left(\frac{\partial\eta}{\partial\theta}+\frac{\zeta}{r}\right)(1-\sigma)+\sigma\left(\frac{\partial\xi}{\partial z}+\frac{\partial\zeta}{\partial r}\right)\right] \tag{3}$$

DYNAMICAL EQUATIONS

Remembering that the p's are forces per unit area, the total force at z in the z-direction is, $p_1 rd\theta \, . \, dr$.

Similarly, the total force at $z+dz$ is, $p_1 rd\theta \, . \, dr \, . + \frac{\partial}{\partial z}(p_1 rd\theta \, . \, dr)dz$.

Hence, the difference in tension on the two faces of the element causing acceleration in the z-direction is, $\frac{\partial}{\partial z}(p_1 rd\theta \, dr)\, dz$.

The net forces radially and tangentially are, likewise,
$\frac{\partial}{\partial r}(p_2 rd\theta \, . \, dz)dr - p_3 d\theta \, . \, dr \, . \, dz$ and $\frac{\partial}{\partial\theta}(p_3 dr \, . \, dz)d\theta$, respectively.

If the above forces be equated to the resulting changes in momentum, we have, after crossing out differentials on both sides of the equations,

$$\frac{\partial p_1}{\partial z}=\rho\frac{\partial^2\xi}{\partial t^2} \tag{4}$$

$$r\frac{\partial p_2}{\partial r}+p_2-p_3=\rho r\frac{\partial^2\zeta}{\partial t^2} \tag{5}$$

$$\frac{\partial p_3}{\partial\theta}=\rho\frac{\partial^2\eta}{\partial t^2}$$

where ρ is the density of the material.

If purely longitudinal and radial vibrations be considered, $\eta=0$, $\frac{\partial^2\eta}{\partial t^2}=0$, and we are left with Equations (4) and (5). In them substitute for p_1 and p_2.

$$\alpha^2\frac{\partial^2\xi}{\partial z^2}+\beta^2\left(\frac{\partial^2\zeta}{\partial r\,.\,\partial z}+\frac{1}{r}\frac{\partial\zeta}{\partial z}\right)=\frac{\partial^2\xi}{\partial t^2} \tag{6}$$

$$\alpha^2\left(\frac{\partial^2\zeta}{\partial r^2}+\frac{1}{r}\frac{\partial\zeta}{\partial r}-\frac{\zeta}{r^2}\right)+\beta^2\frac{\partial^2\xi}{\partial r\,.\,\partial z}=\frac{\partial^2\zeta}{\partial t^2} \tag{7}$$

where,
$$\alpha^2 = \frac{q(1-\sigma)}{\rho(1+\sigma)(1-2\sigma)}.$$
$$\beta^2 = \frac{q\sigma}{\rho(1+\sigma)(1-2\sigma)} \quad .$$

If shear forces had been included, the left-hand side of Equation (6) would contain the term,
$$\gamma^2\left(\frac{\partial^2\xi}{\partial r^2} + \frac{1}{r}\frac{\partial\xi}{\partial r} + \frac{\partial^2\zeta}{\partial r\,.\,\partial z} + \frac{1}{r}\frac{\partial\zeta}{\partial z}\right)$$
and the left-hand side of Equation (7) would contain,
$$\gamma^2\left(\frac{\partial^2\zeta}{\partial z^2} + \frac{\partial^2\xi}{\partial r\,.\,\partial z}\right)$$
where, $\gamma^2 = \frac{q}{2\rho(1+\sigma)}$ = (velocity of shear waves in a solid)^2.

When the above shear terms are included in Equations (6) and (7), as well as the terms in η, these equations agree with those given by Love (8, p. 291).

Solution of Dynamical Equations

Assume as solutions,
$$\xi = A\,f_1(r)\,e^{i\omega(t-z/c_1)}$$
$$\zeta = B\,f_2(r)\,e^{i\omega(t-z/c_1)}$$
where, c_1 = phase velocity, $\omega = 2\pi \times$ frequency; t = time; and $f_1(r)$ and $f_2(r)$ are functions only of r, and A and B are constants.

Substitute the assumed solutions in Equation (6) and differentiate with respect to r. Then multiply by $-\frac{\beta^2}{\alpha^2}$.

Substitute the solutions in Equation (7) and multiply by $\frac{\alpha^2 - c_1^2}{\beta^2}$.

The two equations resulting from the foregoing may now be added together to give,
$$r^2\frac{\partial^2 f_2}{\partial r^2} + r\frac{\partial f_2}{\partial r} + (k^2r^2 - 1)f_2 = 0 \tag{8}$$
where,
$$k^2 = \frac{\alpha^2(\alpha^2 - c_1^2)}{\alpha^2(\alpha^2 - c_1^2) - \beta^4} \cdot \frac{\omega^2}{\alpha^2} \quad . \tag{9}$$

Equation (9) may be re-written as,
$$c_1^2 = \alpha^2\left(1 - \frac{\beta^4}{\alpha^4} \cdot \frac{k^2\alpha^2}{k^2\alpha^2 - \omega^2}\right) \quad . \tag{10}$$

The solution of (8) is, $f_2 = J_1(kr)$, so that,
$$\zeta = B J_1(kr)\,e^{i\omega(t-z/c_1)}.$$

Substituting this value in either of Equations (6) or (7) leads to,
$$\xi = \frac{Bc_1}{i\omega k\beta^2}(k^2\alpha^2 - \omega^2)\,J_o(kr)e^{i\omega(t-z/c_1)}.$$

Boundary Conditions

At $r = a$, $p_2 = 0$.

Substituting the expression for p_2 leads to,
$$(1-\sigma)\frac{\partial\zeta}{\partial a} + \sigma\left(\frac{\partial\xi}{\partial z} + \frac{\zeta}{a}\right) = 0 \ .$$

If now the values for ξ and ζ be used in this equation, and x be written for ka, we have, finally,

$$\frac{J_1(x)}{xJ_o(x)} = \left(\frac{1-\sigma}{1-2\sigma}\right)\frac{4\pi^2a^2f^2}{\alpha^2} \quad . \tag{11}$$

Frequencies of Radial Resonance

Examination of Equation (10) reveals that $\frac{k\alpha}{2\pi}$ has the dimensions of frequency, and that c_1 becomes infinite when $\omega = k\alpha$; that is, anomalous dispersion occurs at this point. By analogy with the results obtained for liquids (5), it may be concluded that $\frac{k\alpha}{2\pi}$ represents the frequencies of resonant radial vibrations in the solid rod.

To determine the values of k appropriate to the conditions, derive Equation (11) again, with $\frac{\partial\eta}{\partial\theta} = \frac{\partial\xi}{\partial z} = 0$; that is, in using the boundary condition, consider pure radial vibrations. Instead of Equation (11), the following equation then results,

$$\frac{(1-\sigma)}{(1-2\sigma)}\, xJ_o(x) = J_1(x) \tag{12}$$

where again $ka = x$.

The frequencies determined by the expression, $\frac{k\alpha}{2\pi}$, agree with those given by Ruedy (12).

Velocity Equation

From Equation (10), if f_r be written for $\frac{k\alpha}{2\pi}$, we have,

$$c_1^2 = \alpha^2\left[1 - \left(\frac{\sigma}{1-\sigma}\right)^2 \frac{f_r^2}{f_r^2 - f^2}\right] \tag{13}$$

since $\omega = 2\pi f$, where f is the frequency, and $\frac{\beta^4}{\alpha^4} = \frac{\sigma^2}{(1-\sigma)^2}$.

II. Derivation of Velocity Equation with Viscosity Included

In order to include viscosity in the analysis, we shall follow the procedure developed by Stokes (13) and consider the viscous forces as being proportional to the time rate of shearing strain (s), that is, equal to $\frac{\mu ds}{dt}$, where μ is the coefficient of viscosity.

In Section I it was assumed that no net shearing strains existed, and it might appear, therefore, that the effect of viscosity would then be zero. However, it may be shown (1, p. 110) that any strain (a simple elongation, for example) may be considered as being compounded of two simple shears and a dilatation. Hence in the following analysis, although the net strains (a, b, c), as in Section I, are purely axial, we shall consider them as being made up of the shears, (e_1, $-e_1$, 0) and (e_2, 0, $-e_2$), and the dilatation (d, d, d).

We have (1, p. 110, Equation (4)), $d=\frac{1}{3}(a+b+c)$, $e_1=\frac{1}{3}(a-2b+c)$ and $e_2=\frac{1}{3}(a+b-2c)$.

If k = volume elasticity and n = modulus of rigidity,

$$\left.\begin{aligned} p_1 &= 3dk + 2n(e_1+e_2) \\ p_2 &= 3dk - 2ne_1 \\ p_3 &= 3dk - 2ne_2 \end{aligned}\right\} \tag{14}$$

On Stokes' assumption, if the coefficient n be replaced by the operator $\left(n+\mu\frac{\partial}{\partial t}\right)$, the contributions of the viscous forces will be fully taken into account (9, p. 560). Remembering that,

$$k = \frac{q}{3(1-2\sigma)}\ , \qquad n = \frac{q}{2(1+\sigma)}\ ,$$

we have,

$$\left.\begin{aligned} p_1 &= \frac{q}{(1+\sigma)(1-2\sigma)}\Big[(1-\sigma)a+(b+c)\sigma\Big] + \frac{2}{3}\mu\frac{\partial}{\partial t}\Big(2a-b-c\Big) \\ p_2 &= \frac{q}{(1+\sigma)(1-2\sigma)}\Big[(1-\sigma)b+(c+a)\sigma\Big] + \frac{2}{3}\mu\frac{\partial}{\partial t}\Big(a-2b+c\Big) \\ p_3 &= \frac{q}{(1+\sigma)(1-2\sigma)}\Big[(1-\sigma)c+(a+b)\sigma\Big] + \frac{2}{3}\mu\frac{\partial}{\partial t}\Big(a+b-2c\Big) \end{aligned}\right\}. \tag{15}$$

In Equations (15), formed after recombination of the two shears and the dilatation, *it will be noticed that the modulus of rigidity, n, does not appear,* as it would if the *net* strains involved shearing. The moduli that do appear are the "elongational" elasticity, $\frac{q(1-\sigma)}{(1+\sigma)(1-2\sigma)}$, $\frac{q\sigma}{(1+\sigma)(1-2\sigma)}$, and the coefficient of viscosity, μ. The forces due to the resolved shears and dilatation add together so that their resultant is the same as was obtained in Section 1 by dealing immediately with the net (purely axial) strains. The forces due to viscosity, however, cannot be combined with any force occurring as the result of dilatation. They therefore appear in each equation of (15) as an extra term. It is the same as though a further shearing force had been added, except that the force due to viscosity is 90° out of phase $\left(\text{on account of the operator } \frac{\partial}{\partial t}\right)$ with a shear force of equal numerical value.

Using the equations for p_1, p_2 and p_3, we proceed exactly as in Section I and find that now,

$$c_1^2 = \frac{[\alpha^2(\omega^2-k^2\alpha^2)+k^2\beta^4+\frac{3}{4}k^2\varphi^2]+\omega^2\varphi}{(\omega^2-k^2\alpha^2)+k^2\varphi}\ , \tag{16}$$

where

$$\varphi = \frac{4}{3}\,\frac{i\omega\mu}{\rho}\ ,$$

and that k is given by the equation,

$$ka\,\frac{J_1(ka)}{J_0(ka)} = \left(\frac{1-\sigma}{1-2\sigma}\right)\frac{4\pi^2a^2f^2}{1-\frac{3\varphi}{2\alpha^2}\left(\frac{1-\sigma}{1-2\sigma}\right)}\cdot\frac{1}{\alpha^2}\ . \tag{17}$$

Equation (16) shows that c_1 is now complex. If $\frac{1}{c_1}$ be denoted by $\frac{1}{c_r} - i\delta$, the real phase velocity will then be c_r, and we shall have the damping term, $e^{-\delta\omega x}$, added to the expressions for the particle velocities.

When the equation for c_r is plotted, Curve 4 results. The surprising thing that will be observed is that the phase velocity is *increased* at high frequencies. One would expect, of course, that viscosity would have the opposite effect. This peculiar result is discussed further in Section III.

III. On the Solution of the Wave Equation with a Viscosity Term Included

In considering the effect of viscosity, Stokes (13) has been followed by Rayleigh (10, p. 315), Lamb (7, p. 186-188) and others in including viscosity as proportional to the time rate of shearing stress. To investigate the effect of such an assumption, it is convenient for a moment to consider simpler equations than Equation (6) or Equation (7) in Section I, and for this purpose we can take the one applying to rods when radial motion is neglected, and include in it a term to take care of viscosity. This equation then is (9, p. 561, Equation (7)),

$$\frac{\partial^2 \xi}{\partial t^2} = c_0^2 \frac{\partial^2 \xi}{\partial x^2} + R \frac{\partial^3 \xi}{\partial x^2 \partial t} \tag{18}$$

where

$$R = \tfrac{4}{3} \frac{(1+\sigma)\mu}{\rho} .$$

Equation (18) is practically identical with that obtained for liquids (7, p. 188, Equation (12)).

As a solution of Equation (18), we follow Rayleigh and Lamb, and assume,

$$\xi_1 = A e^{int + mx} . \tag{19}$$

From (18),

$$-n^2 = m^2 c_0^2 + R m^2 in$$

or,

$$m^2 = \frac{-n^2}{c_0^2 + Rin} . \tag{20}$$

Let $m = a + ib$, and let the right-hand side of Equation (20) be denoted by $iA - B$.

Then, $a^2 - b^2 = -B$, and, $ab = \frac{A}{2}$.

Hence

$$b^2 = \frac{B}{2}\left(1 + \sqrt{1 + \frac{A^2}{B^2}}\right) .$$

The phase velocity, c_1, is given by $\frac{n}{b}$, and therefore,

$$\frac{c_1^2}{c_0^2} = \frac{2(1+\theta^2)}{1+\sqrt{1+\theta^2}} \tag{21}$$

where,

$$\theta = \frac{Rn}{c_0^2} .$$

On account of the viscosity term, θ, Equation (21) shows that the phase velocity, c_1, in a viscous medium will always be *greater* than the phase velocity, c_o, in a frictionless medium. This result can hardly be justified on physical grounds, and might indicate that viscosity has not been introduced correctly into the wave equation, or else that the assumed particular solution is not the correct one to use.

Instead of Equation (19) we may take,

$$\xi_2 = Ae^{mt + ikx} \quad . \qquad (22)$$

Then, $m^2 = -c_o^2k^2 - Rmk^2$.

And, if $m = a + ib$, we have,

$$b = kc_o\sqrt{1 - \frac{R^2k^2}{4c_o^2}} \quad . \qquad (23)$$

Since the phase velocity is given by $\frac{b}{k}$, we have,

$$\frac{c_1}{c_o} = \sqrt{1 - \frac{R^2k^2}{4c_o^2}} \quad . \qquad (24)$$

This expression for c_1 seems more plausible than the preceding one, as it indicates a lower value for the phase velocity than that for the velocity in a frictionless medium. An examination of the two assumed solutions of the wave equation, however, shows that the second solution is physically not very likely. The essential difference between the expression for ξ_1 and the expression for ξ_2 is that in the first case damping is assumed with space but not with time, and in the second case damping occurs with time but not with space. For ξ_1 we have waves going out from the origin with ever-decreasing amplitude as the distance x increases, but at any time the amplitude of the waves at a point in space is the same as the amplitude at the same point at any other time. In the case of ξ_2 the waves from zero to infinity have all the same amplitude, and as time progresses the amplitude of each wave dies away at the same rate.

This latter conception is somewhat artificial and is certainly not true for waves progressing through a viscous medium. It is interesting, however, that it does give a more likely value for the phase velocity than the previous solution. Finally it may be remarked that it appears strange that the phase velocities should be different in the two cases, though it occurs probably on account of the physical unreality of the second assumed solution.

If, instead of taking viscous damping as proportional to the time rate of shearing, it be taken simply as proportional to the particle velocity, the term $R\frac{\partial^3\xi}{\partial x^2 \partial t}$ in the wave equation, (Equation 18) will be replaced by $R\frac{\partial \xi}{\partial t}$. Then both the assumed solutions mentioned above result in values for the phase velocity which are lower than the velocity in a frictionless medium. This result alone, however, is hardly sufficient to justify the simpler method of introducing viscosity. But, in any case, there is something wrong with the classical method, and further investigation of this matter seems to be required.

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RAPID DETERMINATION OF MOISTURE IN GRAIN

I. COMPARISON OF 130°C. AIR OVEN AND BROWN-DUVEL METHODS WITH VACUUM OVEN METHOD[1]

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Abstract

Some 300 samples of hard red spring wheat, 50 of amber durum wheat and 75 each of oats and barley, covering the entire moisture range of commercial importance, were employed in the comparisons. Provision was made for statistical estimation of sampling errors, errors of method and errors of observation. A two-stage drying procedure was employed for the vacuum oven determinations which were taken as the reference standard. Sampling errors, average difference between identical ovens and errors due to daily fluctuations were all larger than the standard deviation of duplicates ("experimental error"), the sampling error being the largest.

A comparison of the Hobart and Wiley grinders shows the latter to be superior for preparing wheat for the 130° C. air oven determination. The air oven consistently underestimates the moisture content of all the grains tested, as compared with the vacuum oven, the deficit increasing with the actual moisture content. This systematic bias may be eliminated by means of a linear correction equation. After allowing for the observed sampling errors and errors of the vacuum oven determination, the net standard error of prediction with the air oven using a Wiley mill is, with hard red spring wheat 0.24%, with amber durum wheat 0.12%, with barley 0.20% and with oats 0.20% moisture.

The Brown-Duvel method also underestimates the moisture content, this deficit also increasing with the actual moisture content in the case of spring and amber durum wheat. After applying a correction equation to eliminate the systematic bias, the net standard error of prediction of moisture by this method was found to be: with hard red spring wheat 0.16%, with durum wheat 0.09%, with barley 0.12% and with oats 0.13%. The Brown-Duvel method, therefore, when carefully operated makes possible a more consistent estimate of the actual moisture content, as determined by the vacuum oven, than does the 130° C. air oven method. As with the vacuum oven, both the 130° C. air oven and the Brown-Duvel appear to be subject to slight variations affecting all the determinations made on any particular day.

I. Introduction

Information respecting the amount of moisture contained in cereals is exceedingly important commercially. As the more accurate laboratory methods for estimating moisture content are ordinarily too slow and cumbersome, many rapid but somewhat empirical methods have been devised to meet the requirements of the grain trade, electrical moisture meters constituting the latest development in this connection. The object of the present investigation was a critical study of the utility and accuracy of a selected number of these rapid methods, including five electrical moisture meters of different make or design. Rapid analytical methods investigated were the Brown-Duvel and the 130° C. air oven. Other rapid analytical methods such as the carbide method for flour described by Blish and Hites (3) and the methyl alcohol method of Cashmore (5) were not tested as they are not in common

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use in the trade. The electrical testers studied consisted of the Burton-Pitt, Davies, Limbrick, hand-operated Tag-Heppenstall and motor-operated Tag-Heppenstall instruments; the results obtained with these meters will be discussed in the next paper of this series.

The procedure in comparing the general reliability of these methods consisted of determining the moisture content of a subsection of some 300 samples of hard red spring wheat, 50 of amber durum wheat and 75 samples each of oats and barley, by each method simultaneously, with provision for statistical estimation of sampling errors, errors of method and ordinary experimental error. Actually the statistical computations were made on a somewhat smaller number of samples in each case, for in an investigation of this size there were a few inevitable losses due to accidents, etc. It was planned originally to include a similar number of rye and flax samples, but these grains proved not to be available in sufficient quantity for a statistical study. Samples of each grain were selected so that about one third of the total number fell into each of the moisture classes, "straight," "tough" and "damp," the exact moisture limits of which are shown in Table I. The grades encountered and the number of samples in each grade were determined by the quality of the grain received at the inspection points during the collection period. In general, and especially with spring wheat, the higher grades were more abundant than the lower ones, but the samples obtained cover the entire range of moisture content and grade normally found in commercial practice.

TABLE I

MOISTURE LIMITS OF STRAIGHT, TOUGH AND DAMP GRAIN*

Kind of grain	Percentage of moisture (by official Brown-Duvel method) allowed		
	Straight	Tough	Damp
Hard red spring wheat	Up to 14.5	Over 14.5 but not over 17.0	Over 17.0
Durum wheat	Up to 14.8	Over 14.8 but not over 17.0	Over 17.0
Oats	Up to 14.0	Over 14.0 but not over 17.0	Over 17.0
Barley	Up to 14.8	Over 14.8 but not over 17.0	Over 17.0
Rye	Up to 14.0	Over 14.0 but not over 17.0	Over 17.0
Flax	Up to 10.5	Over 10.5 but not over 13.5	Over 13.5

**As established by the Board of Grain Commissioners for Canada.*

The vacuum oven was employed as a standard of comparison and two separate subsamples were tested by this method in order to obtain an estimate of the sampling error arising from the subdivision of the samples. In addition, any day-to-day variations in the results by the methods under investigation were studied by subdividing three large samples, one in each of the straight, tough and damp classes, into a large number of subsamples, and daily determining the moisture content of two of these by the vacuum oven method, and that of one of each daily by each of the other methods.

2. Experimental Material

Collection and Storage of Samples

The grain samples used were obtained at various points in western Canada by the staff of the Western Grain Inspection Division. One-gallon samples were collected, placed in moisture-tight cans and shipped to the Dominion Grain Research Laboratory, Winnipeg, where they were cleaned and their approximate moisture content determined. A larger number of samples than required was collected in order to permit selection of the requisite number within each moisture class. Only some 30 samples of naturally damp wheat were received so that over 70 samples had to be artificially dampened by adding water in order to obtain the necessary damp samples. After being dampened with distilled water, the samples thus treated were allowed to stand for one month in moisture-tight cans in a room maintained at a temperature near the freezing point. The samples were removed from the cans every few days, thoroughly mixed and replaced in the cans. The naturally straight, tough, and damp samples were also stored under the above conditions for at least a month, with occasional mixing, before they were subdivided for the various tests. All of the damp samples of oats and barley tested were "naturally" damp when received, but as no naturally damp samples of amber durum wheat were available, certain straight grade and tough samples were moistened by exposure to high humidity for several days, dried back to the required moisture in the laboratory and stored before testing.

Subdivision of Samples

It was necessary to divide each sample into eight subsamples and for this purpose the Boerner (4) sampler, recommended by the American Association of Cereal Chemists (1), would have been extremely slow and laborious. To have superimposed several such samplers one on another would have been unduly expensive. A one-to-eight progressive riffle was therefore designed and constructed. The principle of this apparatus is evident from Fig. 1. The chutes leading from one set of riffles to the next were placed at an angle of 32° to the horizontal. At this angle even excessively damp wheat would flow without stopping and in all cases the kernels spread quite uniformly across the chutes, thus insuring that the receiving riffle was fed uniformly over its entire width.

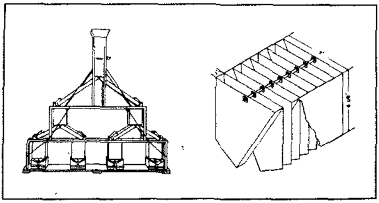

Fig. 1. *Riffle sampler.*

The individual riffle units consisted of eight sections one inch wide, adjoining sections discharging in opposite directions. An adjustment, for setting

each section in an individual riffle unit to receive the same quantity of grain, was provided by passing a threaded rod, with nuts on each side of each section, through the middle of the section in each riffle unit one inch below the top. By means of these nuts the sides of the sections could be adjusted so that each one discharged the same weight of grain. This adjustment was made on each section of each riffle before the sampler was assembled.

As the samples to be used had been kept for a month or more in moisture-tight cans, with periodic mixing, it was assumed that each sample was reasonably homogeneous with respect to moisture. The subdivision of these relatively small samples into eight subsamples appeared therefore to present a relatively simple sampling problem and the apparatus described above, after adjustment to give eight subsamples of practically equal size, was considered adequate for the purpose. Nevertheless, in order to obviate any possible systematic error, the eight spouts of the sampler were numbered and the corresponding subsamples distributed at random among the methods and instruments under test.

In practice the amount of grain sampled was only slightly in excess of that required to fill the eight half-pint double-tight paint cans employed as subsample containers. The sampling was done in a room in which the temperature was near the freezing point, and it was found that exposing even a damp sample for six hours under these conditions did not reduce the moisture content by any amount measurable by the Brown-Duvel method. The samples were divided two or three days before actual use and the resulting subsamples were then allowed to attain temperature equilibrium before testing by letting them stand for 24 hr. in the room in which the determinations were made.

3. Experimental Methods

Preliminary Study of Technique

Preparation of samples for vacuum oven

Samples of whole grain must be ground before their moisture content can be determined by the oven (indirect) methods. Obviously the moisture loss during grinding will depend on the fineness of grinding, the type of grinder employed, the atmospheric conditions in the room in which the grinding is done, and the initial moisture content of the grain. Flohil and his collaborators (8) have already stressed the importance of these points.

The official method of the Association of Official Agricultural Chemists (9) demands that the sample should be ground to pass through 1-mm. circular openings. This procedure is qualified by the statement that if the sample cannot be thus ground it should be reduced to as fine a condition as possible. The method of the American Association of Cereal Chemists (1) requires 30–40 gm. of wheat to be ground to a floury consistency, or to such a degree of fineness that at least 50% of it will pass through a 36 grits gauze sieve. Neither of these descriptions specify the type of mill to be employed for grinding.

Two general types of grinders are available for reducing the size of the material. The attrition type, such as a Hobart mill, is capable of reducing the material, unless of high moisture content, to a fine floury consistency, but it has the disadvantage of heating the sample and thus increasing the moisture loss. The shearing type, such as the Wiley mill, does not heat the sample to nearly the same extent, but it gives a coarser granulation and requires a longer period for grinding. This involves longer exposure to the atmosphere of the grinding room with a resultant differential loss of moisture from samples of different initial moisture. The Brabender electro-magnetic grinder was tested in preliminary experiments, but when it was set for the finest granulation possible the material produced was still quite coarse. Moreover, under these conditions the mill heated appreciably and was impracticably slow.

In order to reduce the moisture loss due to exposure to the atmosphere and any slight loss that might occur from heating in the grinder, a two-stage drying method was employed. The first stage consisted of drying 100.00 ± 0.01 gm. of the wheat to equilibrium with an atmosphere of 40% relative humidity at 72° F. This was done in shallow dishes four by five inches in size. To accelerate the rate of drying during this initial stage, and also to avoid contamination with dust, the samples were conditioned in a ventilated cabinet which consisted essentially of a fan for drawing air over the samples, a series of shelves, a set of adjustable dampers for regulating the air flow, and a screen for excluding dust from the incoming air. A diagrammatic sketch of this cabinet is shown in Fig. 2. No heater or regulators were required as the atmospheric conditions in the room were controlled.

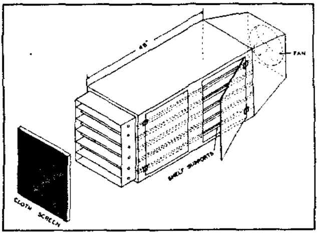

FIG. 2. *Conditioner employed in first stage of drying for vacuum oven determination.*

Under these conditions it was found that even the dampest samples attained approximate equilibrium in 72 hr., all of them then containing about 10.8% moisture. The samples were then re-weighed and ground in the same room, and 30 to 40 gm. of the ground material taken for the vacuum oven determination. The loss in weight during both stages was then computed in terms of moisture and expressed as a percentage of the weight of the original wet sample.

A preliminary experiment was made to compare the results obtained by the vacuum oven method using both the single and two-stage methods with a series of wheat samples of different initial moisture content. One portion of each sample was ground in a Hobart and one in a Wiley mill to compare the two methods of grinding. The Hobart mill was set to grind the material as finely as possible without choking, and a 1-mm. sieve was employed in the Wiley mill, since this meets the requirement of the official vacuum oven method (9). The results are given in Table II.

TABLE II

PERCENTAGE MOISTURE INDICATED BY VACUUM AND AIR OVENS USING DIFFERENT GRINDERS AND METHODS OF DRYING

Sample No.	Vacuum oven, 5½ hr.		Vacuum oven, 16 hr.		130° C. Air oven, 1 hr.	
	Wiley mill	Hobart grinder	Wiley mill	Hobart grinder	Wiley mill	Hobart grinder
Wheat ground directly (single-stage method)						
1	11.22	10.28	11.45	10.38	11.18*	11.66
2	13.42	13.46	13.65	13.60	13.45	13.86
4	16.15	16.30*	16.48	16.51*	16.43	16.52
5	18.37	18.13	18.76	18.30	18.64	18.70
Wheat dried to equilibrium with atmosphere before grinding (two-stage method)						
1	11.40	11.56	11.88	11.68	11.70	11.86
2	13.64	13.69	14.02	13.96	13.76	13.96
4	16.48	16.16	16.77	16.31	16.72	16.46
5	19.02	18.68	19.42	18.72	19.19	18.90

**Single determination only; duplicate lost.*
All other figures are the average of duplicate determinations.

In practically all cases, and especially with samples of high moisture content, the two-stage method shows higher moisture content than the single-stage method in which the wheat is ground directly. It seems unlikely that the additional respiration during the preliminary drying could account for a difference of this magnitude, and it is concluded that there is a moisture loss during grinding which justifies the use of the two-stage method for the vacuum oven determinations that are to be used as a standard of comparison. On the whole, samples ground in the Wiley mill show higher moisture content than those ground in the Hobart grinder, indicating that the heating which occurs in the latter causes a greater loss of moisture than that occasioned by the longer exposure necessary for grinding in the Wiley mill.

Time of heating in vacuum oven

Comparison of the moisture contents, given in Table II, after 5½ and 16 hours' heating in the vacuum oven shows quite definitely that the shorter period does not reduce the samples to constant weight. The vacuum oven was operated throughout in accordance with the official methods (1, 9) which require heating to constant weight, qualified by the statement "approximately 5 hours." Bailey (2) also points out, as the result of a collaborative study, that in many cases constant weight is not attained by five hours' drying. The period of heating required to give constant weight was therefore subjected to further test. Five samples of different initial moisture content were studied, using the single-stage method, each determination being made on triplicate portions of approximately 2.0 gm. These were removed from the vacuum oven at intervals varying from 5 to 20 hr. Twenty minutes was allowed for releasing the vacuum and the dishes were allowed to cool for 20

min. over calcium carbide before weighing. On replacing the dishes in the oven, 15 min. was allowed for evacuation and restoration of temperature equilibrium. The average moisture content obtained after each of the intervals is plotted in Fig. 3 (solid line).

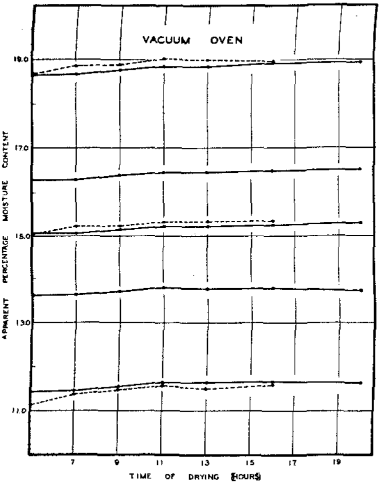

FIG. 3. *Influence of time of drying in vacuum oven on apparent moisture content of hard red spring wheat.*

Another experiment of this type was made using a slightly different procedure. Three samples were employed and 18 portions of approximately 2.0 gm. weighed out from each. These were all placed in the oven at the same time and three replicates from each sample removed at 5-, 7-, 9-, 11-, 13- and 16-hr. intervals respectively, cooled for 20 min. over CaC_2, weighed, and the apparent moisture content computed. The oven was closed immediately after the removal of each set of samples, and in computing the time of subsequent withdrawals, ten minutes was allowed for re-evacuation each time the oven was opened. The results obtained are also shown in Fig. 3 (dotted line).

The results of the first experiment show that the two samples of lowest initial moisture content had reached constant weight, over a two-hour interval, after 11 hours' heating, while the samples of high moisture content lost weight throughout the entire 20-hr. drying period. In the second experiment, in which selected samples only were removed at each time interval, and the oven immediately closed and evacuated, constant weight was reached in an 11-hr. heating period. The procedure used in the second experiment was doubtless more accurate and representative of the conditions employed in making moisture determinations. As a result of this study a 16-hr. drying period was employed throughout the comparative studies.

Grinding of samples for 130° C. air oven

The rapid air oven method for grain, as described in the official methods of the Association of Official Agricultural Chemists (9) requires the heating of the material for two hours in an oven at 135° ± 2° C. It is not stated how the material is to be ground. This Association and the American Association of Cereal Chemists both recommend drying for one hour at 130° ± 3° C.

in an air oven as a rapid method for flour. The air oven method is not recognized as an official one for grain, but according to Coleman (6) it is commonly employed. Determination of its suitability for this purpose therefore was one of the objects of this investigation.

As this method is to be employed as a rapid determination it is obviously out of the question to use the two-stage drying procedure employed with the vacuum oven samples. This again brings up the problem of grinding the grain, especially when damp. Furthermore, since the method was originally developed for flour it might be expected to be applicable only to finely ground material.

This method was therefore subjected to a preliminary experiment using further portions of the samples employed in the preliminary tests with the vacuum oven. Both the Hobart and Wiley grinders were used in combination with single- and two-stage drying methods. The results obtained are shown in Table II.

In these few preliminary experiments the air oven and the vacuum oven methods gave similar results on material treated in the same way. As the two-stage procedure is not permissible for a rapid method, the higher moisture contents indicated by it are of little consequence, except that one can predict that the rapid air oven method (single-stage) will give lower results than a two-stage vacuum oven method. The findings are indefinite as to whether the Wiley mill or Hobart grinder is the better for the 130° C. air oven method when the samples are ground directly. It was decided therefore to grind one half of the main series of 300 hard red spring wheat samples, referred to earlier, in the Wiley mill and the other half in the Hobart grinder, thus enabling the errors inherent in both methods to be determined as well as permitting comparisons with the vacuum oven results.

TECHNIQUE FINALLY ADOPTED

Vacuum oven

As a result of the preliminary experiments already described, a two-stage method of drying was adopted. The vacuum oven technique employed was that described in the official methods (1, 9), using a 16-hr. drying period at a temperature of 99 to 100° C. and a pressure of 25 mm., or less, of mercury. Two De Khotinsky vacuum ovens, the vacuum chambers of which are immersed in oil, were employed, each oven being provided with a Hyvac pump. Metal dishes with inside fitting covers as required in the official methods (1) were used, the covers being loosened but not removed while drying proceeded in the oven. Calcium carbide was used in the desiccators in which the dishes were cooled.

Air oven

No preliminary drying was employed in this determination. The ground sample was dried at 130° C. ± 3° C. for one hour, the officia' method for flour (1, 9). A size *C* De Khotinsky drying oven was used. Twenty dishes were placed in the oven at a time, these being all on one shelf. The thermometer bulb was at the same level as the dishes. Dishes with inside fitting

covers were employed, the covers being entirely removed during drying. Approximately 2-gm. samples were employed. The desiccators used were charged with calcium carbide.

Brown-Duvel

The Brown-Duvel tester has been fully described by Coleman and Boerner (7), but as special refinements are practised in the Dominion Grain Research Laboratory, where these tests were made, and in the offices of the Western Grain Inspection Division generally, the conditions found necessary to secure uniformity of operation will be outlined in some detail.

In accordance with the customary procedure, all thermometers, distillation flasks, measuring cylinders and condensing tubes were checked for accuracy of dimensions as specified by Coleman and Boerner (7), and in addition the thermometers were standardized between 160° C. and 195° C. by the Division of Physics, National Research Council of Canada. The calibrations of the measuring cylinders were checked within the range 10.5 to 17.0 cc. and those with an error greater than ± 0.1 cc. were discarded.

It has been found in the Dominion Grain Research Laboratory that the time required for heating and cooling has a decided effect on the results. The tester used was a six compartment machine manufactured by the Seed Trade Reporting Bureau, Chicago, but the heating elements were made in Winnipeg, to specifications prepared by the Laboratory. These required the individual heaters to give 675 ± 7 watts at normal line voltage. A separate transformer and heavy leads were used to prevent a voltage drop of over 2% when the testers were all in operation. Voltage controllers are employed at many of the Western Grain Inspection offices, but these were not installed in connection with the equipment used in this investigation. The line voltage, however, was checked frequently during the tests by means of a voltmeter mounted permanently above the tester. The heating rate in all compartments, as determined by the oil test, was finally rendered uniform by adjusting the distance of the heating element from the flask. The heating time employed for the oil test was 25 min. rather than 20 min. as specified by Coleman and Boerner.

In order to obtain a uniform cooling time the tester was located in a position protected from draughts, in a room held at approximately 21° C. by means of a thermostat. As the cooling time is also affected by the distance the heating plate falls away from the heating chamber, a set screw was provided on the swivel arm to regulate this distance. All compartments were thus adjusted so as to cool the flasks to 160° C. in approximately 20 min. when testing wheat of 14.5% moisture. Determinations on actual samples under examination were only made after the machines had been put into condition by a preliminary test. The oil used was at room temperature and sufficient was maintained to accommodate a full day's test, in order to avoid the use of warm oil returned from the tests to storage. The "cut-off" temperatures employed were as follows: hard red spring wheat 180° C.; amber durum wheat 180° C.; barley 190° C.; and oats 195° C.

4. Vacuum Oven Results

Hard Red Spring Wheat

Two of the subsamples, obtained from the subdivision of each sample, were dried to equilibrium at 72° F. and 40% relative humidity, and ground. Duplicate 2-gm. portions were taken from each for the vacuum oven determinations. Comparison of the results obtained from the two subsamples made possible an estimate of the sampling error.

As two ovens were required to accommodate the number of samples tested daily, a study of the difference between ovens was made possible, by placing one of the duplicates of each subsample in each of the two ovens. The two De Khotinsky vacuum ovens already described were operated under exactly the same conditions of temperature and pressure.

The 1200 individual results obtained from the three hundred wheat samples were subjected to an analysis of variance, the total variance being divided into portions attributable to average difference between ovens, differences between subsamples and the difference between duplicates after eliminating any average difference between ovens. The need for determining the sampling error in an investigation of this type was demonstrated by the fact that the variance between subsamples was significantly greater than that between duplicates of the same subsample, even in this relatively simple sampling process, which merely consisted of subdividing a small sample. There was also a significant difference between ovens, which demonstrates that ovens of identical construction, operated under the same conditions, may give slightly different results. The results of the foregoing analysis, expressed in terms of percentage of moisture, are summarized in Table III.

TABLE III

Results of analysis of variance of vacuum oven determinations

Grain	Statistic	Moisture, %
Hard red spring wheat	Average difference between ovens	0.071
Hard red spring wheat	Standard error of duplicates ("experimental error")*	0.044
Hard red spring wheat	Standard error of sampling	0.110
Hard red spring wheat	Standard error of duplicates plus sampling error*	0.118
Hard red spring wheat	Day-to-day variations (standard deviation)	0.084
Amber durum wheat	Standard error of duplicates plus sampling error*	0.093
Barley	Standard error of duplicates plus sampling error*	0.089
Oats	Standard error of duplicates plus sampling error*	0.109

**Standard errors apply to mean of duplicates.*

Two subsamples of a large sample in each of the straight, tough, and damp classes, were analyzed daily by all methods, as previously mentioned, with a view to ascertaining whether the methods exhibited daily variations.

As the experiments with wheat were continued over a period of eleven days, the results should give a fairly reliable indication of the variations that might be encountered in practice. The daily fluctuation of the observed vacuum oven moisture content of each sample is shown graphically in Fig. 4.

The correlation between fluctuations in apparent moisture content of all three samples from day to day, is evident from the diagram, and is confirmed by an analysis of variance, which shows that the variation due to daily fluctuation, common to all three samples, is significantly greater than the residual variance attributable to sampling errors, experimental errors, etc. After allowing for these errors, the net variance attributable to daily variation corresponds to a standard error of 0.084% moisture, which is greater than the standard error of duplicates given in Table III.

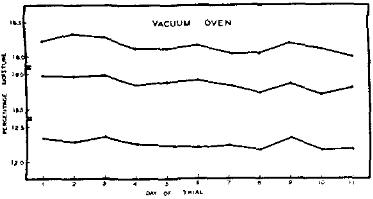

FIG. 4. *Daily fluctuation in moisture content by vacuum oven method (hard red spring wheat).*

DURUM WHEAT, BARLEY AND OATS

The vacuum oven experiments with these grains were conducted in exactly the same manner as were those with hard red spring wheat, and the results were subjected to the same statistical analysis. Instead of 300 samples of each, however, only about 50 samples of amber durum wheat, 75 of barley, and 75 of oats were available. Towards the close of this phase of the investigation, it was found that the results secured from one of the ovens were affected by the position of the sample in the oven, portions of samples placed on the bottom shelf showing a moisture content as much as 0.25% lower than further portions of the same samples placed on the top shelf. The results obtained from this oven had, therefore, to be discarded. As two out of the four replicates of each sample were thus lost, it was impossible to separate the standard error of the duplicates from the sampling error, an estimate of the combined errors only being obtained. The standard error of the duplicates obtained from spring wheat may, however, with reasonable assurance be applied to these grains. The combined sampling and experimental errors for the different grains are given in Table III.

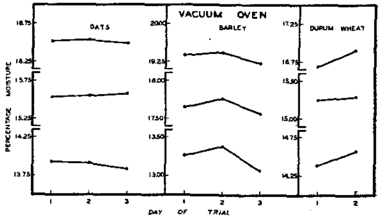

FIG. 5. *Daily fluctuation in moisture content by vacuum oven method (durum wheat, barley and oats).*

As the number of samples of durum wheat, barley and oats was much smaller than that of common wheat, the tests with any one grain were completed within two or three days. The moisture content of the three samples tested daily by the vacuum oven is shown in Fig. 5. There is some evidence of a systematic daily fluctuation in the results with barley and durum wheat, but the number of days available is too small to obtain a reasonably reliable estimate of the variance.

5. Air Oven Results

HARD RED SPRING WHEAT

It was felt at the outset that the 130° C. air oven would probably be the most accurate of the rapid methods, and in order to avoid a sampling error, possibly large enough to vitiate an estimate of its accuracy in comparison with the vacuum oven, the portions required for the former method were taken from one of the subsamples subsequently employed for the two-stage

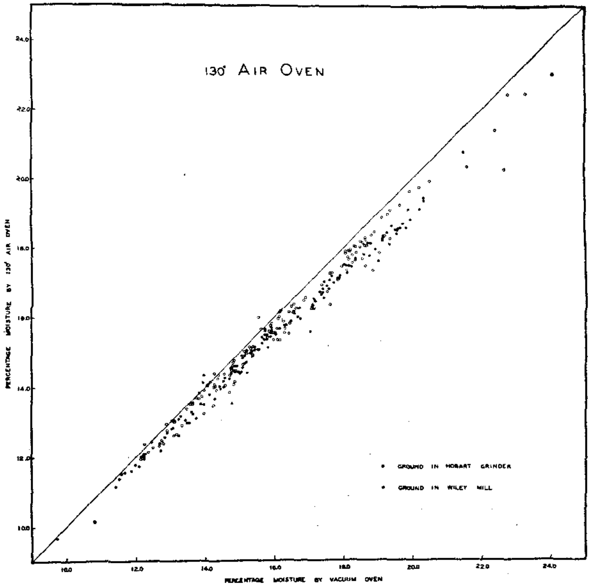

FIG. 6. *Moisture content of hard red spring wheat samples by 130° C. air oven and vacuum oven methods.*

vacuum oven determinations. Statistical comparisons were thus made between the air oven and the vacuum oven on the same subsample, so that the sampling error was virtually eliminated.

As previously mentioned, it was impossible to decide from the preliminary experiment whether the Hobart grinder or Wiley mill was best suited for the air oven method and in consequence 150 of the 300 wheat samples were ground in the Hobart and the other 150 in the Wiley mill.

The results obtained by both methods are shown in Fig. 6, from which it is evident that the Wiley mill has given more consistent results than the Hobart grinder. It is also evident that the air oven method, regardless of the

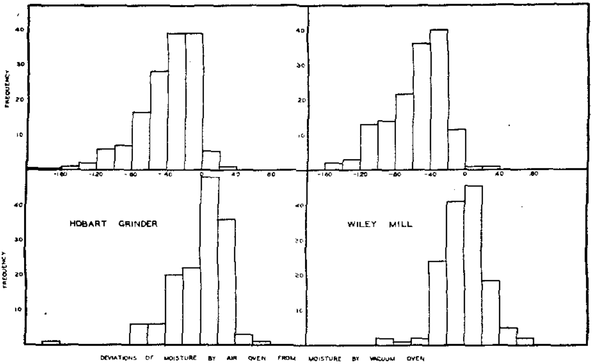

Fig. 7. Frequency distribution of deviations of moisture by 130° C. air oven from moisture by vacuum oven (hard red spring wheat). Upper portion, observed deviations; lower portion, deviations after application of correction equation.

method of grinding employed, consistently underestimates the amount of water present, and that the extent of this underestimation increases with the absolute moisture content. The results already presented in Table II suggest that the consistent underestimation of the moisture by the air oven can be attributed to the single-stage method of drying. This may also account in part for the increasing deficit with damp samples, although it is conceivable that the air oven is incapable of removing all of the moisture from such samples in the one-hour drying period.

The frequency distribution of the deviations of the observed moisture content by the air oven method, from the corresponding vacuum oven result, is shown for both methods of grinding in the top two histograms of Fig. 7. A distinct skewness is evident; most of the deviations are on the negative side, some being in defect by as much as 1.6% moisture. This systematic bias was eliminated by calculating by the method of Least Squares, a linear

regression equation expressing the expected vacuum oven moisture in terms of the observed air oven result, for both methods of grinding. These equations, together with the standard error of duplicates, the standard error of prediction of the vacuum oven values after applying the correction equations, and the net standard error of prediction due to differences in the results by the two methods, after allowing for the observational error in the vacuum oven determination, are shown in Table IV. In these equations the regression coefficients, by which the air oven results must be multiplied, give a measure of the tendency toward greater underestimation as the moisture content increases. The frequency distributions of the deviations of the air oven from the vacuum oven results, after applying this linear correction, are shown in the lower half of Fig. 7. It is evident that these distributions are now much more symmetrical, with the mode quite close to the zero value. The determinations made on material ground in the Hobart grinder, however, still exhibit a greater degree of asymmetry than those employing the Wiley mill.

TABLE IV

STANDARD ERROR OF DUPLICATES, LINEAR CORRECTION EQUATIONS AND OBSERVED AND NET STANDARD ERRORS OF PREDICTION OF VACUUM OVEN PERCENTAGE MOISTURE CONTENT OF HARD RED SPRING WHEAT BY THE 130° C. AIR OVEN USING TWO GRINDING METHODS

Grinder employed	Standard error of duplicates	Correction equation*	Standard error of prediction	
			Observed	Net
Hobart	0.069	$M_V = -0.644 + 1.066\ M_A$	0.31	0.30
Wiley	0.050	$M_V = -0.926 + 1.095\ M_A$	0.25	0.24

*M_V = *moisture content as determined by vacuum oven.*
*M_A = *moisture content as determined by air oven.*
Standard errors apply to mean of duplicates.

The standard error of the duplicates, commonly taken as a measure of the "experimental error" in the air oven method was higher than the value found for the vacuum oven (0.044%) by both methods of grinding, the Hobart grinder giving a larger error than the Wiley mill. It is noteworthy, however, that compared with this value the standard error of prediction of vacuum oven moisture by the air oven method is relatively large. This indicates that although duplicates may check fairly well within themselves, there may be a relatively large discrepancy in predicting the moisture content by the vacuum oven method from results obtained in the air oven. The net standard error of prediction, after allowing for the variance of duplicates in the vacuum oven, is not significantly smaller than the observed standard error. This is to be expected since the air oven and vacuum oven determinations were made on the same subsamples, as explained earlier, and the results are not affected by differences between subsamples. The results in Table IV show that the standard errors of prediction, and of the duplicates, are lower with the Wiley than with the Hobart grinder, showing the former mill to be superior for this determination.

The results obtained from the three samples tested daily by the air oven method to detect the existence of daily variation are shown in Fig. 8 for both methods of grinding. Upon analysis these do not reveal consistent daily fluctuations in the results obtained from all three samples. Nevertheless, in view of the known existence of daily variation in the vacuum oven results, it seemed desirable to investigate the effect of eliminating daily differences. These differences in both the vacuum and air oven results may be eliminated from comparisons of the two methods by taking the variances and covariance about the daily means, instead of about the general mean of the entire series. When this is done the standard error of prediction, using the Wiley mill, is reduced from 0.24% to 0.23% moisture, but the standard error of prediction, using the Hobart grinder, previously most variable, is reduced from 0.30% to 0.22% moisture. The reason for this reduction is apparent in Table V, which shows the average difference between the moisture determinations by the air oven and vacuum oven on different days. The Hobart grinder exhibits a variation in this respect of no less than 0.52% moisture as compared with 0.29% in the case of the Wiley mill, and a maximum difference of 0.24% in the daily series of vacuum oven results (Fig. 4). The observed irregularity of the Hobart results (Fig. 6) would thus appear to be due to the difficulty of maintaining an identical setting of the machine from day to day. The elimination of daily variations by the above method reduces the errors of prediction, but this lower value is only applicable to comparisons of the two methods on a particular day. The errors of prediction, given in Table IV, are those applicable to the air oven method with hard red spring wheat, as used in ordinary practice.

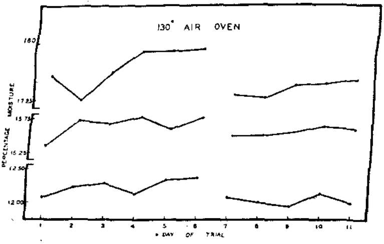

FIG. 8. *Daily fluctuation in moisture content by 130° C. air oven method (hard red spring wheat). Days 1–6, Hobart grinder; days 7–11, Wiley mill.*

TABLE V

AVERAGE DIFFERENCE BETWEEN PERCENTAGE MOISTURE BY 130° C. AIR OVEN AND MOISTURE BY VACUUM OVEN BY DAYS

Day	Hobart grinder	Day	Wiley mill
1	−0.72	7	−0.59
3	−0.55	8	−0.63
4	−0.46	9	−0.61
5	−0.22	10	−0.34
6	−0.20	11	−0.56

Effect of grade

Only a small number of low-grade samples was encountered. Thirty-seven of these, grading No. 4 Northern, No. 5, No. 6 or Feed, were selected for comparison with an equal number of high-grade (No. 1 Hard and No's 1, 2 or 3 Northern) samples of approximately the same moisture content. Owing

to the use of two grinders, and the pronounced day-to-day variations affecting results obtained when the Hobart grinder was used, valid comparisons could be made only between tests performed on the same day. The variance and covariance of vacuum oven and air oven moisture about the daily means were computed for high- and low-grade samples separately and collectively. An analysis of variance indicates that neither the regression of vacuum oven moisture on air oven moisture nor the standard error of prediction differed significantly in the two cases.

Results with artificially dampened samples

In view of the length of time elapsing between the tempering of the artificially dampened samples and the performance of the tests it seemed unlikely that these would be differentiated in any way from naturally damp samples. Nevertheless, previous findings by Coleman (6) indicated the desirability of comparing the behavior of artificially dampened samples in the electrical moisture meters with that of naturally damp grain, and this study was extended to include all methods. Somewhat surprisingly there did appear to be a significant difference with the air oven, the underestimation of the moisture being less with artificially dampened samples by about 0.25% moisture on the average. The majority of the naturally damp samples available were, however, tested on one day, on which there were no artificially dampened ones; and when the straight and tough samples, tested on the same day, were compared with the remainder of the straight and tough, these also exhibited a significant differentiation in the same direction. It appears likely, therefore, that the observed difference between natural and artificially dampened samples may be due partly, if not wholly, to daily fluctuations, of the type previously noted in the results by the vacuum oven, air oven, or both.

Durum Wheat, Barley and Oats

The investigation of the air oven method with these grains was conducted in exactly the same manner as that employed with hard red spring wheat; the air oven sample being obtained from one of the vacuum oven samples to avoid the sampling error. As the Wiley mill gave the lowest error of prediction with spring wheat this grinder was the only one employed in connection with these other grains.

The results obtained by the air oven as compared with the vacuum oven for all three grains are shown in Fig. 9. It is obvious that they exhibit the same characteristics as those obtained with spring wheat, namely, a consistent underestimation of the moisture content which increases as the moisture increases. This systematic bias was eliminated, as before, by calculating in the same manner a linear regression equation for each of the three grains. The standard error of prediction of the observed vacuum oven values was computed, and also the error of prediction after eliminating the "experimental error" of the vacuum oven determinations. As one pair of duplicates was lost owing to the failure of the vacuum oven, only the combined error of the vacuum oven and the sampling error was obtained, so that in making this

correction, where no sampling error is involved, it was necessary to use the "experimental error" of the vacuum oven obtained from the results with hard red spring wheat. The formulas for the linear correction equations, the standard errors of prediction and the standard errors of duplicates, are given in Table VI for the three grains. The regression coefficients are again

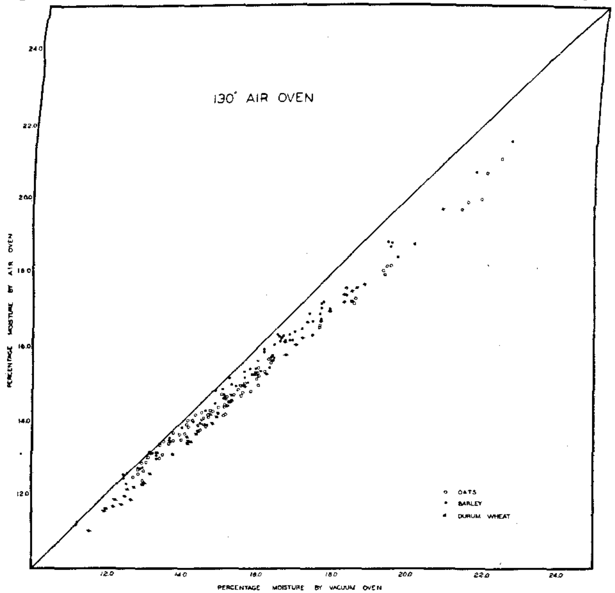

FIG. 9. *Moisture content of durum wheat, barley and oat samples by 130° C. air oven and vacuum oven methods.*

TABLE VI

STANDARD ERROR OF DUPLICATES, LINEAR CORRECTION EQUATIONS, AND OBSERVED AND NET STANDARD ERRORS OF PREDICTION OF VACUUM OVEN PERCENTAGE MOISTURE OF AMBER DURUM WHEAT, BARLEY AND OATS, BY THE 130° C. AIR OVEN

Grain	Standard error of duplicates	Correction equation*	Standard error of prediction	
			Observed	Net
Amber durum wheat	0.052	$M_V = -0.452 + 1.084\ M_A$	0.13	0.12
Barley	0.056	$M_V = -1.548 + 1.131\ M_A$	0.21	0.20
Oats	0.057	$M_V = -2.225 + 1.195\ M_A$	0.21	0.20

*M_V = *moisture content as determined by vacuum oven.*
*M_A = *moisture content as determined by air oven.*
Standard errors apply to mean of duplicates.

all greater than unity, since this method shows an increasing tendency to underestimate the moisture content as the actual water content increases.

It is evident from Table VI that the standard error of the duplicates ("experimental error") is about the same for all three grains, being only slightly larger than that given in Table IV for common wheat ground in the Wiley mill. The standard error of prediction for all of these grains, however, is lower than the corresponding value for hard red spring wheat. There are two possible explanations for this behavior: either the air oven method is capable of more nearly reproducing the vacuum oven results with the other grains than with spring wheat, or else the analysts became more skilled during the course of the investigation, since these grains were studied last. It seems improbable, however, that the latter possibility accounts for the entire difference, since all of the men were skilled analysts, and since a similar result was observed with the Brown-Duvel method, the operator of which had been making the determination continuously for years.

The results obtained from the amber durum, barley and oat samples employed in the study of daily variations are shown in Fig. 10. As fewer samples of these grains were tested, than in the case of hard red spring wheat, the results do not extend over a period of more than three days at the most. Again the three individual samples yield no conclusive evidence of daily variation, but as before, the situation with respect to the results as a whole was investigated by computing the variances and covariance around the daily means instead of the general mean. When this was done the standard error of prediction for amber durum wheat was unchanged, that for barley was reduced from 0.20% to 0.15% and that for oats from 0.20% to 0.17%. These results demonstrate the extent to which daily variations affected the comparisons of the two methods. The larger error is the one applicable to ordinary routine determinations.

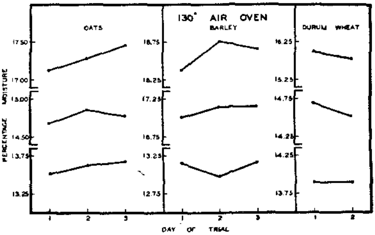

FIG. 10. *Daily fluctuation in moisture content by 130° C. air oven method (durum wheat, barley and oats).*

6. Brown-Duvel Results

HARD RED SPRING WHEAT

The Brown-Duvel determination was made according to the technique previously described, using a separate subsample of grain. In comparing this method with the vacuum oven, allowance must be made for both the experimental error of the vacuum oven determination and errors of sampling.

The results obtained by the Brown-Duvel method are plotted against the corresponding vacuum oven results in Fig. 11. They show essentially the same behavior as did the air oven results, namely, an underestimation of the moisture content which increases as the moisture increases. In this instance, however, the phenomenon is believed to be due to an entirely different set of causes, since the grain had not been ground. The consistent

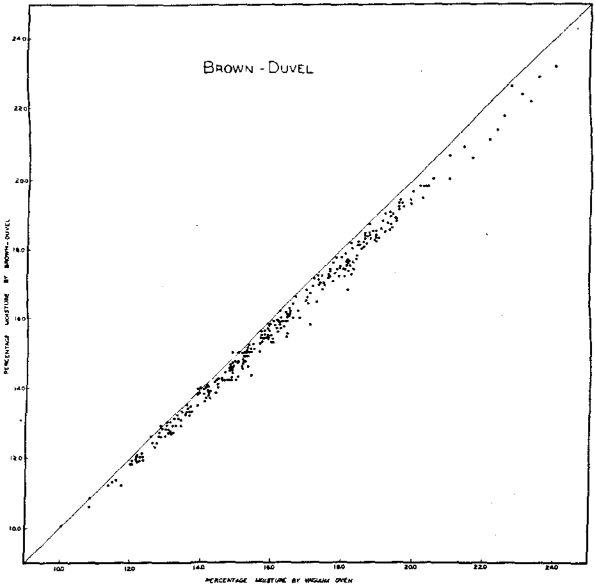

FIG. 11. *Moisture content of hard red spring wheat samples by Brown-Duvel and vacuum oven methods.*

underestimation of the moisture content is easily explained by the fact that the Brown-Duvel method was originally developed to give results comparable with the old water-jacketed oven drying method, which in turn is known to give lower moisture values than the vacuum oven. It is also known (1) that a cut-off temperature of 190° C. gives moisture values more nearly equivalent to those of the vacuum oven than a cut-off temperature of 180° C. which is the one commonly used for wheat, and employed in this investigation.

Although the consistent underestimation by the Brown-Duvel method may thus be readily accounted for, these considerations can scarcely explain

why the extent of the underestimation should become progressively greater as the moisture content increases. This may be due to variation in the heating time with grain of different moisture content. It has been shown by Coleman and Boerner (7) that slow heating gives low results while rapid heating gives high results. As the time required to attain the desired temperature increases with the moisture content of the grain, even when the heaters are carefully standardized by the oil test, it is obvious that a differential deficit is to be expected.

The frequency distribution of the deviations of the observed moisture content by the Brown-Duvel from that by the vacuum oven method is shown in the upper part of Fig. 12. A distinct negative bias is observable, but on the whole the deviations show a more symmetrical distribution than those of the air oven results. This systematic bias was eliminated by computing a linear regression equation as before. This equation, together with the standard error of prediction of the observed vacuum oven values and the standard error of prediction after allowing for the sampling error and experimental error of the vacuum oven method are given in Table VII. The frequency distribution of the deviations after applying the correction equation is shown in the lower part of Fig. 12. The increase in accuracy is evident.

Table VII shows that the standard error of the duplicates in the Brown-Duvel method is practically the same as that obtained using the Wiley mill in conjunction with the air oven, these being respectively 0.053% and 0.050%. The standard error of prediction of the vacuum oven results, however, is much lower for the Brown-Duvel than for the Wiley mill and the air oven. The net standard error of prediction attributable to discrepancies between the methods is much less than the observed error, owing to the fact that the latter includes errors of sampling.

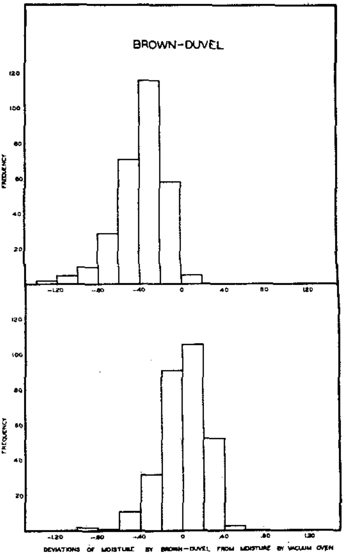

FIG. 12. *Frequency distribution of deviations of moisture by Brown-Duvel from moisture by vacuum oven (hard red spring wheat). Upper portion, observed deviations; lower portion, deviations after application of correction equation.*

TABLE VII

STANDARD ERROR OF DUPLICATES, LINEAR CORRECTION EQUATIONS, AND OBSERVED AND NET STANDARD ERRORS OF PREDICTION OF VACUUM OVEN PERCENTAGE MOISTURE BY THE BROWN-DUVEL METHOD WITH HARD RED SPRING WHEAT, AMBER DURUM WHEAT, BARLEY AND OATS

Grain	Standard error of duplicates	Correction equation*	Standard error of prediction	
			Observed	Net
Hard red spring wheat	0.053	$M_V = -0.280 + 1.042M_{BD}$	0.21	0.16
Amber durum wheat	0.040	$M_V = 1.026M_{BD}$	0.15	0.09
Barley	0.042	$M_V = 0.046 + 1.005M_{BD}$	0.17	0.12
Oats	0.034	$M_V = 0.674 + 0.983M_{BD}$	0.16	0.13

*M_V = *moisture content as determined by vacuum oven.*
*M_{BD} = *moisture content as determined by Brown-Duvel.*
Standard errors apply to mean of duplicates.

These results show that the Brown-Duvel method, when used by a skilled operator and all precautions are taken to ensure accuracy, is superior to the air oven method, using the best grinder tested, for estimating the moisture content of hard red spring wheat as determined by the vacuum oven method. Both methods, however, underestimate the moisture content, but for different reasons, and the application of a linear correction equation is required to estimate the true moisture content as determined by the vacuum oven method.

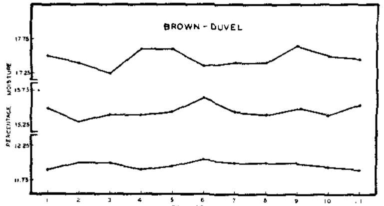

FIG. 13. *Daily fluctuation in moisture content by Brown-Duvel method (hard red spring wheat).*

The three samples of common wheat tested daily by the Brown-Duvel method (Fig. 13) show no evidence of systematic daily fluctuation. Nevertheless the standard error of prediction was reduced from 0.16% to 0.13% moisture by calculating the variances and covariance around the daily means instead of the general mean, indicating that day-to-day variations have been a definite source of error in this comparison of the vacuum oven and Brown-Duvel methods.

Effect of grade

As in the case of the 130° C. air oven, the results obtained with the small number of low-grade samples encountered did not differ significantly from those of the high-grade samples selected for comparison.

Results with artificially dampened samples

With artificially dampened samples a similar method of analysis to that applied to the air oven observations again gave similar results, using the vacuum oven as standard. There appeared to be a significant difference

between natural and artificially dampened samples, but differences in the same direction were also obtained by contrasting the results with straight and tough samples, on the three days on which naturally damp samples occurred, with those of the remaining straight and tough samples. This occurrence is in fact common to all methods and instruments tested, strongly suggesting daily variations in the vacuum oven as the underlying cause.

Durum Wheat, Barley and Oats

The results for all three grains are presented in Fig. 14. It is evident that the discrepancies between the vacuum oven and Brown-Duvel results are considerably smaller than those obtained with hard red spring wheat; durum wheat showing the greatest departures. The linear equations, calculated as before, for estimating the vacuum oven moisture value from the Brown-Duvel determination for each of the grains, are given in Table VII. Application of these correction equations eliminates the systematic bias inherent in

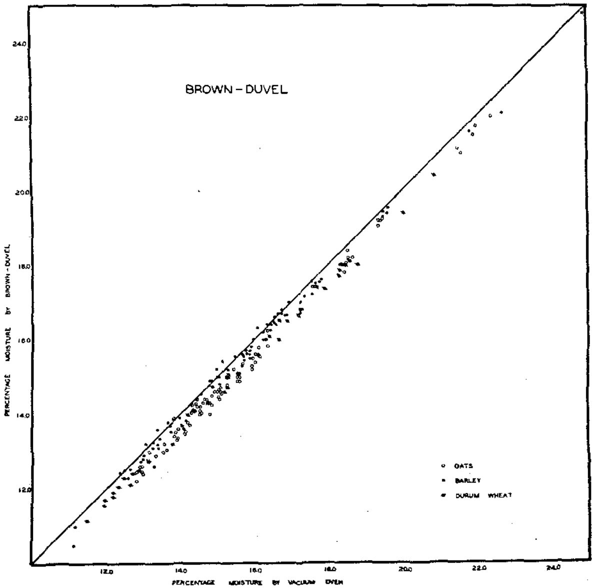

FIG. 14. *Moisture content of durum wheat, barley and oat samples by Brown-Duvel and vacuum oven methods.*

the method. The regression coefficient is in all cases close to unity, showing that this method maintains a practically uniform differential throughout the moisture range studied.

It would appear that the cut-off temperatures recommended for these grains yield results in closer agreement with the vacuum oven method than the cut-off temperature recommended for common wheat. The tendency of the method toward increased underestimation with increasing moisture content is evident only with durum wheat. As this behavior is not shown by oats and barley, it is possible that a variation in the heating time has less effect on the results with these grains. Presumably the presence of the hull on oats and barley removes part of the complicating effect of decomposition.

The observed standard errors of prediction of the vacuum oven results by the Brown-Duvel, and the net errors after allowing for the experimental error of the vacuum oven method and the sampling error, are given in Table VIII together with the standard errors of the duplicate determinations. Comparison of the errors of the duplicates with the corresponding values for common wheat shows that the Brown-Duvel determination is somewhat more accurate when dealing with the coarse grains. The standard error of prediction is likewise less than that obtained with hard red spring wheat, a result in harmony with the air oven findings. It appears therefore that both the air oven and Brown-Duvel are more accurate for coarse grains than for common wheat. Comparison of the air oven with the Brown-Duvel shows the latter to be superior for predicting the moisture content, as determined by the vacuum oven, in all grains tested. The "experimental error" of both methods is about the same in all cases, the Brown-Duvel being slightly better than the air oven with coarse grains. Both methods underestimate the moisture content and the results require correction by means of a linear regression formula to give results comparable with those obtained by the vacuum oven.

The results obtained from the three samples tested daily during the study of coarse grains are plotted in Fig. 15. These do not show any systematic daily variation, but when the variances and covariance of the experimental samples are calculated around the daily means instead of the general mean, the net standard error of prediction for durum wheat is reduced from 0.09% to within the sampling error, and for barley from 0.12% to 0.11%. The reduction in the case of oats affects only the third decimal place.

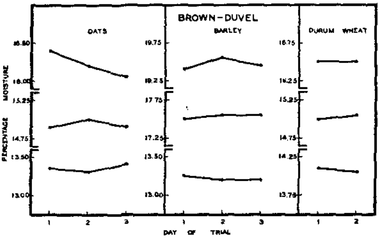

FIG. 15. *Daily fluctuation in moisture content by Brown-Duvel method (durum wheat, barley and oats).*

7. Summary and Conclusions

Vacuum oven

1. A double-stage drying procedure, in which wheat was dried to equilibrium with the room atmosphere before grinding, was found to give higher moisture values than those obtained when the grain was ground directly. The former procedure was therefore used in conjunction with the vacuum oven determinations.

2. When the vacuum oven used was filled with samples, at least 11 hours' heating was required for these to reach constant weight over a two-hour interval. A 16-hr. drying period was therefore employed for all experimental determinations.

3. Two vacuum ovens of identical construction, operated under the same conditions of temperature and pressure, gave an average difference of 0.071% moisture between 300 pairs of duplicate samples of hard red spring wheat, one oven yielding consistently slightly higher results than the other.

4. After eliminating the average difference between ovens the standard error of the mean of duplicates ("experimental error") was 0.044% moisture with hard red spring wheat and the daily fluctuations, computed from three pairs of samples tested daily, resulted in a standard deviation of 0.084% moisture. The experimental error thus forms only a part of the variance encountered in the vacuum oven method and is not an accurate measure of the precision of determinations made by it.

5. The relatively simple sampling operation of dividing a small amount of grain into eight equal subsections resulted in a sampling error of 0.110% moisture with hard red spring wheat. This was the limiting factor determining the precision of the vacuum oven, and the accuracy of the various rapid methods tested. It is evident that in investigations of this type, an estimate of the sampling error must be made if the correct conclusions are to be drawn from comparisons of different methods.

6. The combined experimental and sampling errors encountered with other grains were: amber durum wheat 0.093%, barley 0.089%, and oats 0.109%.

130° C. air oven

7. Both Hobart and Wiley grinders were employed in conjunction with the 130° C. air oven method; the latter was found to be more accurate as shown from the standard error of prediction given in Table VIII. The greater error with the Hobart grinder was traced to greater daily fluctuations.

8. The air oven method was found to underestimate the moisture content, as determined by the vacuum oven, of all grains studied, the deficit increasing with the actual moisture content. The consistent underestimation is believed to be due to evaporation during grinding, as in the former method the grain was ground without preliminary drying. This procedure may also account in part for the increase of the deficit with moisture content, but it is also conceivable that the air oven is incapable of removing all of the moisture from damp samples in a one-hour drying period.

Brown-Duvel

9. The Brown-Duvel method was also found to underestimate the moisture content of most of the grains studied; and with hard red spring and amber durum wheat, the deficit increased with the actual moisture content.

TABLE VIII

"EXPERIMENTAL ERROR" AND STANDARD ERROR OF PREDICTION OF VACUUM OVEN PERCENTAGE MOISTURE BY AIR OVEN AND BROWN-DUVEL METHODS

Determination	Standard error of duplicates ("Experimental error")	Net standard error of prediction
Air oven		
Hard red spring wheat		
Hobart grinder	0.07	0.30
Wiley mill	0.05	0.24
Amber durum wheat (Wiley)	0.05	0.12
Barley (Wiley)	0.06	0.20
Oats (Wiley)	0.06	0.20
Brown-Duvel		
Hard red spring wheat	0.05	0.16
Amber durum wheat	0.04	0.09
Barley	0.04	0.12
Oats	0.03	0.13

Standard errors apply to mean of duplicates.

10. It is evident from Table VIII that the Brown-Duvel method is capable of giving a more consistent estimate of the moisture content as determined by the vacuum oven, than is the 130° C. air oven. As the Brown-Duvel determination is more dependent on the skill of the operator and on a close adherence to the specified conditions, than the air oven determination, this result might not apply in general practice, particularly if Brown-Duvel equipment operated with gaseous or liquid fuel had to be used. The above tests, however, can be taken as typical of the Brown-Duvel determination as used in the offices of the Western Grain Inspection Division.

11. The standard errors of prediction given in Table VIII indicate that the air oven and Brown-Duvel methods are capable of greater accuracy with coarse grains than with wheat.

Daily fluctuations

12. The wheat samples analyzed daily to study the extent of the daily fluctuations in methods, show systematic variations in the vacuum oven, and in the air oven when the material was ground in a Hobart grinder. The results obtained by the remaining methods with wheat and with the coarse grains by all methods, did not in general give clear-cut results, but the standard errors of prediction were in most cases reduced by calculating the variances and covariance around the daily means. This method of computation removes any bias due to daily fluctuations in either the vacuum oven or the method under test. It seems probable, therefore, that small daily variations

occurred in several of the series of tests, but owing to the greater residual variation they could not be demonstrated as clearly as in the case of the vacuum oven.

Acknowledgments

The authors wish to acknowledge the ready assistance of the staffs of the Western Grain Inspection Division, who collected and cleaned the samples, and in particular that of Messrs. W. Chivers and R. Forrester of the Winnipeg Inspection Office, who subdivided the samples. Acknowledgment is also due to Mr. B. Frisell, Mr. F. H. Lehberg and Mr. A. T. Capewell of the Dominion Grain Research Laboratory, who were responsible for the vacuum oven, air oven and Brown-Duvel determinations respectively. Mr. S. Stein, Research Assistant, Associate Committee on Grain Research, ably assisted with the various oven determinations.

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THE CANADIAN SPECIES OF THE TORTRICID GENUS *PERONEA*[1]

By J. McDunnough[2]

Abstract

Forty-three species of the Tortricid genus *Peronea* are dealt with, comprising all the species known to occur in Canada at the present time and including practically all of the North American ones. Characters found in both the male and female genital organs have been used as a means of specific differentiation and there are eight sets of figures illustrating these organs. The range of variation in color and pattern of the individual species is briefly discussed and records of distribution and larval food-plants are given as far as present knowledge permits. Seven species new to science are described.

Introduction

The numerous North American species of the Tortricid genus *Peronea* have long been in need of a thorough revision. Owing to the extraordinary variability of color and pattern in the individual species and the superficial similarity of distinct species, misidentifications have been the rule rather than the exception; to increase the difficulties a number of species are common to both the Nearctic and Palaearctic regions and the attempts to apply the older and valid European names to our North American forms have often resulted disastrously, and increased, rather than improved, the synonymic tangles.

It is only of recent years, thanks to the studies of Pierce, Filipjev, and Sheldon, that the Palaearctic species have become thoroughly understood, and these studies have definitely proved the enormous value of characters found in both the male and female genital organs as a means of delimiting the individual species and correctly associating the numerous forms, both of color and pattern, that have heretofore proved so perplexing. The present paper is an attempt to place our Nearctic, and more particularly our Canadian, species on a similar sound nomenclatorial basis, by the use of characters found in the structure of the genitalia. The material studied has been largely that contained in the Canadian National Collection, but the opportunity has also presented itself, through the courtesy of the collectors, of working over the specimens in the collections of Mr. K. Bowman of Edmonton, Alta., and Mr. W. R. Buckell of Salmon Arm, B.C., thereby increasing notably our knowledge of the range of distribution of a number of species. Considerable valuable material from the Kearfott collection was also made available for study through the co-operation of the authorities of the American Museum of Natural History in New York City, to whom sincere thanks are due. While it has been impossible in most instances actually to examine existing type material, every care has been used in making the specific identifications, based on a detailed study of the original descriptions and such figures as were available. In doubtful cases photographs of the types were secured or speci-

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Contribution from the Division of Systematic Entomology, Entomological Branch, Department of Agriculture, Ottawa, Canada.
[2] *Chief, Division of Systematic Entomology.*

mens sent for comparison to museum specialists, and in this connection the writer wishes to acknowledge gratefully the assistance of Mr. W. H. T. Tams of the British Museum of Natural History, Mr. A. Busck of the United States National Museum and Dr. E. T. Cresson of the Academy of Natural Sciences, Philadelphia. With one or two exceptions, which will be noted in the text, it is felt that the specific determinations are reasonably sure of being correct.

As the genus *Peronea* is comprised essentially of species indigenous to temperate zones, it has not been surprising to find that of all the described North American species, there are only two which as yet have not been found to occur in our Canadian fauna; these are *foliana* Wlshm., a species from the southwestern States and *comariana* Fern., a small species from the Atlantic States which is entirely unknown to the writer. Even with our present limited records the range of distribution of the individual species is very extensive and it is fairly safe to predict that most of the species will be found to extend in range across the entire Dominion.

No attempt has been made at this time to create names for any of the individual color forms and pattern variations, as has been done in Europe. The aim of this paper is merely to present constant structural characters whereby the species may be differentiated, and it is felt that the study of the variation within any one given species may be safely left for some future time when more material, especially in long bred series, is available for study. Further it has been found inadvisable to apply subgeneric names in the group as has been attempted by various European workers; the characters used to define these subgenera appear to the writer to be largely specific rather than subgeneric and several instances occur, as will be noted in the text, where a species links up with two so-called subgenera.

Peronea emargana Fabr.

Page 325, fig. 1; Page 329, fig. 1.

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Acalla emargana Kennel, Palaearct. Tort. 65, Pl. III, figs. 1-4. 1909.
Rhacodia emargana Pierce and Metcalfe, Genit. Brit. Tort. 23, Pl. IX. 1922; Sheldon, Entomol. LXIII, 121. 1930; Filipjev, Ann. Mus. Zool. Acad. Sci. U. R. S. S. 524, Pl. XXV, fig. 2, Pl. XXX, fig. 5. 1930.

The species is known in our North American lists under the name *caudana* Fabr. and is readily recognized by the strong excavation of the costal margin of the forewing. The writer has seen no Nearctic specimens of the typical form with yellow basal area on primaries, all our specimens being rather uniformly dark gray-brown or ruddy-brown with (in eastern Canadian specimens at least) a red streak along the inner margin; this, according to Sheldon, is typical *caudana*. While the genitalia of Canadian specimens do not match exactly with the figures given by Pierce, they do coincide much more closely with those of Filipjev and until, therefore, the matter can be straightened out by European students, it seems best to list the species under the above name.

European writers separate the species from *Peronea* under the generic term *Rhacodia* Hbn. on the strength of the slightly subfalcate forewings with excavated costal margin and in the genitalia, according to Filipjev, (*op. cit.* p. 501), the strong, apical, downward projection of the male valva and the narrow band-like nature of the signum in the female bursa. As regards these characters, the writer is of the opinion that they are of individual, rather than of generic, value; *maccana* has essentially the same type of male genitalia with the exception of a very different aedeagus, but has the normal wing-shape and has always been included in *Peronea*. Filipjev, it is true, suggests (p. 502) that a new subgenus may be needed for this species along with *schalleriana (logiana)* and *sponsana*, but both of the latter species show much more divergence in male genitalia from *maccana* than does *maccana* from *emargana*. Several other species following *maccana* in our present arrangement show more distant relationships with the *emargana* group, as can readily be seen by a reference to the figures. The safest plan, unless one is willing to create a multiplicity of doubtful subgenera, seems to be to include all species under the one generic heading, as noted in the introduction.

Genitalia, Male (Two slides examined). Tegumen without cristae; apical portion of anal sheath rather hood-like, with slight transverse ventro-apical projection; socii long, very narrow, upright; claspers strongly narrowed apically, sacculus bent upward toward costa beyond half, then recurved, ending bluntly in a strong hair-tuft, preceded by a deep excavation, apex of valvula long, pointed, extending above costal margin; aedeagus short, scarcely bent, broad at base, narrowed somewhat apically with a small ventro-apical spine, no cornuti.

Genitalia, Female. Ostium plate narrow, lateral lobes wide apart, moderately long, narrowed rather suddenly apically with small inner projection; ostium slightly asymmetrical, situated on cephalic margin of plate; ductus bursae entirely membranous, long, narrow; signum a small, narrow, scobinate strip of chitin.

Distribution. Ontario, Ottawa, Biscotasing; Alberta, Banff, Calgary, Nordegg; British Columbia, Victoria.

Food Plant. *Salix, Populus, Betula* (in Europe).

Peronea maccana Tr.

Page 325, fig. 2; Page 329, fig. 2.

Teras maccana Treitschke, Schmett. Eur. X (3), 133. 1835.
Acalla maccana Kennel, Palaearct. Tort. 74, Pl. IV, figs. 19, 20. 1909.
Peronea maccana Pierce and Metcalfe, Genit. Brit. Tort. 21, Pl. VIII (*partim*, ♀ *nec* ♂). 1922; Sheldon, Entomol. LXIII, 222. 1930; Filipjev, Ann. Mus. Zool. Acad. Sci. U. R. S. S. 502. 1930.
Teras fishiana Fernald, Trans. Am. Ent. Soc. X, 66. 1882; Forbes, Mem. 68, Cornell Univ. Agr. Exp. Sta. 483. 1924.

A larger species than *hastiana* and readily separated by the very striking form of the male genitalia. Characteristic also is the rigidly oblique line from costa of forewings before half to inner margin at two-thirds, with its edging

of blackish, or reddish-black, slightly raised scales, separating the pale antemedian band from the outer area which is generally largely suffused with red-brown, purple-brown or black-brown, especially in costal section.

The type of clasper in the male genitalia is essentially that of *emargana* Fabr. and the socii also show a relationship; the aedeagus is however of a quite dissimilar type. There are a few very slight, minor differences between the genitalia of European and North American specimens, but scarcely sufficient to warrant the retention of the name *fishiana* Fern. even in a varietal sense.

Genitalia, Male (Two slides examined). Very large, with the claspers narrowed and bent inwards apically. Tegumen broad, without cristae; anal sheath narrowed apically with a strong, pointed, ventro-apical spine; socii long, narrow, erect; claspers narrowed apically, sacculus bent strongly upward toward costa at half, then just as strongly recurved forming a deep excavation and terminating in a curved, blade-like process, beyond which is a strong apical hair-tuft: apex of valvula long, conical, extending above costal margin; aedeagus very long and thin, vesica with a terminal bundle of about six minute cornuti.

Genitalia, Female (Two slides examined). Ostium plate with very broad, long, lateral lobes with a minute spine on each inner edge apically; ostium situated near centre of plate, narrow, semicircular; ductus bursae with the narrow, proximal, chitinized portion extending to just beyond the cephalic margin of ostium plate; membranous portion of ductus expanded proximally and with traces of irregular chitinization, then narrowing to a long, thin tube; bursa small, flask-shaped; signum minute.

Distribution. Quebec, Meach Lake, Aylmer; Ontario, Ottawa, Biscotasing; Alberta, Edmonton; British Columbia, Vancouver.

Food Plant. *Vaccinium* (in Europe).

Peronea ptychogrammos Zell.

Page 325, fig. 6; Page 329, fig. 3.

Teras hastiana var. *ptychogrammos* Zeller, Verh. zool. bot. Gesell. Wien, 213. 1875.

This small species, confused by Zeller and all subsequent workers with *hastiana*, has in reality nothing to do with this species; on genitalic characters it is quite distinct and occupies a unique position in the genus with no very close allies; the claspers show certain similarities with those of *emargana* and *maccana* and for this reason it is given its present position.

The color of the forewings is light gray with a moderately distinct red-brown costal triangle; the most distinctive features of the maculation are a *small black basal dash* just below costa and fine black lines along the cubital and anal veins and along the inner edge of the costal triangle.

Genitalia, Male (Two slides examined). Tegumen broad apically and rather chunky; anal sheath unarmed, short; socii weak, small, roughly circular; sacculus lightly sinuate, terminating in a large raised circular tuft of coarse hairs, the inner edge of which is produced downward to form *a long sharp spine;* aedeagus short, thin, vesica armed with a stout apical spine and two pairs of very small cornuti.

Genitalia, Female (One slide examined). Ostium plate small, but with well-developed lateral lobes, directed slightly outward; ostium on cephalic margin of plate, straight; chitinized portion of ductus bursae short, rectangular; membranous portion enlarged somewhat proximally to a small sack which contains a lightly chitinized plate; signum hemispherical, scobinate.

Distribution. Manitoba, Aweme; British Columbia, Salmon Arm.

Food Plant. Unknown.

Peronea nigrolinea Rob.

Page 325, fig. 3; Page 329, fig. 5.

Teras nigrolinea Robinson, Trans. Am. Ent. Soc. II, 281, Pl. VII, fig. 67. 1869.
Teras ferruginiguttana Fernald, Trans. Am. Ent. Soc. X, 65. 1882.

The name was based on several specimens from Grimsby, Ont. (Pettit); two of these, from the collection of the American Museum of Natural History, have been available for study through the courtesy of the authorities and one of them has been chosen as "lectotype" by the writer; four specimens from Ottawa in the Canadian National Collection match this type excellently. The species is a large one with light gray forewings strongly roughened with numerous scale-tufts; as stated by Robinson, the black line from base to apex of wing is not always sharply indicated, in fact, its presence is probably of rather rare occurrence.

In the western provinces a form with similar genitalia occurs in which the primaries are of a much deeper blue-gray color, variably mottled and suffused with smoky shades and frequently with small sub-basal and median dark patches in the fold; rarely slight ochreous or ruddy tinges occur near base and apex of wing. Following a recommendation by Mr. A. Busck of the United States National Museum, the name *ferruginiguttana* Fern. may be applied to this in a varietal or racial sense; Mr. Busck informs me that all that remained of the unique female type of *ferruginiguttana* from Colorado, when the Fernald collection was taken over, was a single hindwing. His conception of the species is based on other Colorado specimens which agree in genitalia with the form occurring in the western Canadian provinces. The male genitalia are extraordinarily dissimilar to those of any *Peronea* species except the following one; there is probably a certain affinity, in the shape of the claspers, to *maccana* Tr. and through this species to the *emargana* group, but this association is not confirmed by other details in these organs, notably the shape of the socii and aedeagus.

Genitalia, Male (Two slides examined). Tegumen broad, without cristae; anal sheath rather pointed at apex, without any definite armature; socii somewhat decumbent, roughly oval in shape; sacculus drawn out into a long point before half and then curving costad sharply and strongly, the apical section again bent downward and *bluntly truncate*, the large ventral excavation thus formed being roughly circular in outline; apical tuft strong, raised; apex of valvula projecting upward beyond margin of costa; aedeagus long, stout at base, then tapering, strongly bent; vesica armed with a large, stout apical spine and a cluster of four or five small cornuti.

Genitalia, Female. Ostium plate rather narrow, lateral lobes moderately long, wide apart and directed outward with a slight incurve at apex; ostium wide, sinuate; ductus bursae with the chitinous portion moderately long and *obliquely cut off* distally, with the inception of the ductus seminalis immediately beyond this portion; membranous section of ductus long, broad, of slightly varying width; bursa contracted somewhat before fundus with strong stellate signum.

Distribution. Typical form. Ontario, Ottawa, Grimsby. Var. *ferruginiguttana.* Manitoba, Aweme; Alberta, Whitla, High River, Edmonton; British Columbia, Salmon Arm.

Food Plant. Unknown; possibly *Populus.*

Peronea maximana B. & B.

Page 325, fig. 4; Page 329, fig. 6.

Peronea maximana Barnes and Busck, Cont. Nat. Hist. Lep. N. Am. IV (3), 216, Pl. XXXII, fig. 1. 1920.

Very similar to the variety *ferruginiguttana* of the preceding species in size, shape and coloration of forewings. In general the forewings appear much smoother and are more evenly dark blue-gray with less smoky shading; they present further a slightly strigate appearance, due to the presence of very fine, broken dark lines on the veins; in one specimen there is a distinct dark streak from base to apex of wing.

Genitalia, Male (Two slides examined). Very similar in general characters to *nigrolinea.* Socii somewhat smaller; sacculus *truncate* at half, not drawn out to a long point; apex, however, produced to a *long curved spine,* with smaller hair-tuft; apex of valvula shorter; vesica with an increase in the number of small cornuti.

Genitalia, Female (One slide examined). Lateral lobes of ostium plate broader and closer together than in *nigrolinea;* ostium closer to caudal margin of plate and openly V-shaped; chitinized portion of ductus bursae very long, narrowed distally and with a V-shaped terminal incision.

Distribution. Ontario, Biscotasing, Bobcaygeon; Alberta, Edmonton; British Columbia, Kaslo, Salmon Arm, Duncan.

Food Plant. *Populus balsamifera,* according to Bobcaygeon rearings; apple, according to data on Kaslo specimen.

Peronea caliginosana Wlk.

Page 325, fig. 7; Page 329, fig. 4.

Teras caliginosana Walker, Cat. Lep. Het. Brit. Mus. XXVIII, 309. 1863.

This perfectly good species has been heretofore confused with *hastiana,* following an erroneous reference by Walsingham; a tentative identification, made after a study by the writer of a photograph of the type, was confirmed by Mr. W. H. T. Tams of the British Museum to whom specimens for comparison with this type were sent.

The species is one of our largest *Peroneas* and the decidedly mottled nature of the hindwings, combined with the rather even deep purple or gray-brown color of the forewings and an obsolescent maculation renders it easily recognizable; the few western specimens examined show a tendency toward a paler, more grayish, ground-color. The type of male genitalia is distinctly that of the *latifasciana* group.

Genitalia, Male (Three slides examined). Tegumen without cristae; anal sheath unarmed; socii *large*, *erect*, broad, reaching beyond apex of tegumen; sacculus with prominent truncate projection from lower margin apically, preceded by a deep, narrow emargination, hair-tuft short, broad; aedeagus moderately long and wide, vesica armed with a bundle of 8–10 short cornuti.

Genitalia, Female (Two specimens examined). Ostium plate with long, narrow, lateral lobes, pointed apically; ostium broad, sinuate, situated near cephalic margin of plate; chitinized portion of ductus bursae, long, broad, extending as far as the ends of the lateral lobes; membranous portion of ductus broad, narrowed somewhat at half, the inception of the ductus seminalis near bursa which is rather small and armed with a large, wedge-shaped, spined signum.

Distribution. Nova Scotia, Digby; Quebec, Meach Lake; Ontario, Ottawa; Alberta, Edmonton; British Columbia, Kaslo, Salmon Arm, Vancouver, Victoria.

Food Plant. Unknown.

Peronea latifasciana Haw.

Page 325, fig. 5; Page 329, fig. 7.

Tortrix latifasciana Haworth, Lepid. Brit. 414. 1811.
Acalla schalleriana Kennel (*nec* Linn.), Palaearct. Tort. 90, Pl. V, figs. 31-34. 1909.
Argrotoxa schalleriana Pierce and Metcalfe (*nec* Linn.), Genit. Brit. Tort. 17, Pl. VII. 1922.
Phylacophora schalleriana Filipjev, Ann. Mus. Zool. Acad Sc:. U. R. S. S. 508. 1930.
Peronea latifasciana Sheldon, Entomol. LXIV, 30. 1931.

This species has been generally known as *schalleriana* Linn., but Sheldon has shown that the latter name must be applied to the species heretofore known as *logiana*, which leaves the name *latifasciana* available for the species under discussion.

In North America the species occurs in both the typical form, *i.e.*, with broad pale oblique band across the entire forewing and ruddy-brown outer area (Kennel, fig. 32), and the form *labeculana* Freyer (Kennel, fig. 31) in which only a large costo-apical triangle of either a red-brown or black-brown color is present. The male genitalia fit in excellently with Pierce's figure (as *schalleriana*), showing the faint spiculation of the anal sheath and the same shape of the sacculus emargination; in the female the shape of the lateral lobes of the ostium plate shows a slight difference from Pierce's figure in all three specimens examined, the bases being abruptly broadened, but this feature may occur in British specimens when sufficient material is available for study.

It should be noted that the species going under the above name, which is so frequently intercepted in the larval or pupal state on azaleas imported from Holland and Belgium, appears to show constant, slight genitalic differences which may indicate specific or at least racial distinctness. The genitalia agree remarkably well with Pierce's figures of *comparana* Hbn.; in the male the anal sheath lacks spiculation, the sacculus emargination is rectangular and the aedeagus is distinctly smaller and without any cornuti (Pierce's text states "5 small and 1 larger cornuti," but the figure does not show them). Until our European workers are able to clear up this apparent confusion the azalea pest may be known as *latifasciana* var. *comparana* Hbn. The male aedeagus figured by Filipjev (*op. cit.* Pl. XXV, fig. 2) does not fit in with any of the slides examined nor with Pierce's figures and is apparently that of some other species.

Genitalia, Male (Three slides examined). Tegumen broad; anal sheath with a distinct ventro-apical spine and finely spiculate; socii very large, erect, extending beyond apex of anal sheath; sacculus narrowly emarginate beyond half, the outer edge of emargination produced downward to form a fine spine; aedeagus very short, broad and only slightly bent, vesica armed with six to eight small apical spines arranged in two groups.

Genitalia, Female (Three slides examined). Ostium plate with moderately long, parallel lobes, broadening suddenly at base; ostium broad, almost straight, situated on cephalic margin of ostium plate; ductus bursae rather short and entirely membranous, enlarged slightly near base and containing a lightly chitinized plate; signum absent.

Distribution. Quebec, Meach Lake; Ontario, Ottawa, Trenton; Alberta, Edmonton.

Food Plants. *Salix*, *Vaccinium* (in Europe).

Peronea schalleriana Linn.

Page 325, fig. 9; Page 329, fig. 9.

P. Tortrix schalleriana Linnaeus, Syst. Nat. X. 1758.
Peronea viburnana Clemens, Proc. Acad. Nat. Sci. Phil. 347. 1860; Forbes, Mem. 68, Cornell Univ. Agr. Exp. Sta. 485. 1924.
Teras viburnana Robinson, Trans. Am. Ent. Soc. II, 281, Pl. VII, fig. 66. 1869.
Teras tristana var. *famula* Zeller, Verh. Zool. Bot. Gesell. Wien, XXV, 214. 1875.
Acalla logiana Kennel (*nec* Linn.), Palaearct. Tort. 77, Pl. IV, figs. 34-38. 1909.
Eclectis logiana Pierce and Metcalfe (*nec* Linn.), Genit. Brit. Tort. 18, Pl. VII. 1922.
Peronea schalleriana Sheldon, Entomol. LXIII, 195. 1930.

The unfortunate confusion in the nomenclature of this species has been satisfactorily cleared up by Sheldon and henceforth the *Viburnum*-feeder must be known under the name of *schalleriana* Linn. The names *viburnana* Clem. and *famula* Zell. have been used for our North American forms, but the writer cannot see that these differ in any marked respect from the European forms for which there are already prior names available; the range of variation is excellently shown in Kennel's figures.

Genitalia, Male (Two slides examined). Tegumen narrowed apically; anal sheath truncate apically, unarmed; socii long, erect, projecting beyond

end of tegumen; sacculus with a projecting arm at half; aedeagus rather small, tapering, vesica armed with a bundle of 15–20 small cornuti.

Genitalia, Female (One slide examined). Ostium plate with long, narrow, lateral lobes, slightly incurved apically; ostium simple, narrow, situated just above cephalic margin of plate; ductus bursae entirely membranous, rather short and narrow, of even width throughout, inception of ductus seminalis near proximal end; signum a narrow piece of scobinate chitin.

Distribution. Ontario, Bobcaygeon, Pt. Pelee; British Columbia, Salmon Arm.

Food Plant. *Viburnum.*

Peronea oxycoccana Pack.

Page 325, fig. 8; Page 329, fig. 8.

Tortrix oxycoccana Packard, Guide Study Ins. 334. 1869; *id.*, 17th Ann. Rep. Sec. Mass. Brd. Agric. 239. 1870.

As noted in the original description, the forewings of the species have a smooth, shiny look which should be sufficient to separate it from some of the dark forms of *minuta* Rob. which are superficially somewhat similar. The species is quite variable in coloration of forewings; in a majority of specimens these are evenly deep black-brown with scattered ruddy scaling; at times, however, they may be rather bright red-brown and specimens occur in which the predominant scaling is light gray. In the paler specimens a ruddy brown, broken, costal, triangular patch is distinctly visible. The genitalia are remarkably close to those of the preceding species.

Genitalia, Male (Four slides examined). Very similar to those of *schalleriana*, with the same erect socii and a sacculus arm at half which, however, is slightly narrower and more pointed; aedeagus longer and more attenuated apically, vesica with a slightly different arrangement of cornuti, which consist of two longer ones and a bundle of 8–10 short ones.

Genitalia, Female (Three slides examined). Similar to *schalleriana*, but ostium plate higher, more nearly square, with shorter lateral lobes; ostium much as in *schalleriana;* ductus bursae very short and faintly chitinized throughout with inception of ductus seminalis nearer the bursa; signum as in *schalleriana*.

Distribution. Ontario, Constance Bay (near Ottawa); Manitoba, Aweme; Alberta, Edmonton.

Food Plant. Cranberry and probably other *Vaccinium* species.

Peronea variana Fern.

Page 325, fig. 10; Page 329, fig. 10.

Teras variana Fernald, 5th Rep. U.S. Ent. Comm. 847. 1890.
Teras angusana Fernald, Can. Ent. XXIV, 178. 1892.

This well known economic pest, the larva of which is known as the black-headed bud-worm, has caused considerable damage at various times to our Canadian forests of spruce, balsam and hemlock, notably in Cape Breton Island and British Columbia.

The moth well deserves its name as there seems no end to the countless variations of color and pattern it may assume; in this respect it quite equals

the European *hastiana* and its variations occur along similar lines. The scope of the present paper does not permit of a detailed discussion of the variation, but it might be well to note that while the superficially very dissimilar *angusana* is placed in the synonymy of the species on account of the practical similarity of genitalia, this particular variation occurs apparently only very rarely among the adults from spruce- and balsam-feeding larvae; on the other hand, a long series in our collections, showing only a moderate amount of variability, was beaten from cedar at Lyn, Ontario; the reddish-brown ground color (paling at times to a yellow-brown) was present in all specimens and the main variability occurred in the distinctness of the streak from base to apex and its bordering white shade. There is the possibility that *angusana* is an incipient species, the larva of which has become restricted to cedar as a food-plant.

The genitalia combine characters present in several distinct groups of *Peronea* and it is quite possible that the species is a comparatively primitive one.

Genitalia, Male (Three slides examined). Tegumen with the terminal lobes widely separated, with indications of cristae; anal sheath *bifid* at tip, a character only found in *hudsoniana;* the lower lateral points of the sheath are united by a thickened membrane to the sides of the tegumen, the whole forming a hood-like structure; socii *drooping*, produced distally; sacculus with ventral margin produced apically to a sharp spine preceded by a narrow emargination as in the *latifasciana* (*schalleriana* Auct.) group; aedeagus short and stout, as in the same group; vesica with a single short spine.

Genitalia, Female (Two slides examined). Very distinct. Ostium plate with long, narrow lobes, wide apart, each terminating in a sharp spine, inwardly directed; the caudal region of this plate (upper, in figure) is more heavily chitinized and strongly excavated in median section, forming a species of flap which seems to protect the terminal end of the long, narrow, flask-shaped ostium; the other end of the ostium protrudes considerably between the lateral lobes of the ostium plate; ductus bursae entirely membranous, broadly distended at its inception, narrowed greatly at half its length; bursa very small, without signum.

Distribution. Across the entire Dominion of Canada, following the spruce belt; typical *angusana* only known from Ottawa region and Lyn, Ontario.

Food Plants. White spruce, balsam, hemlock; *angusana* probably on cedar.

Peronea chalybeana Fern.

Page 326, fig. 1; Page 330, fig. 1.

Teras chalybeana Fernald, Trans. Am. Ent. Soc. X, 65. 1882.
Peronea chalybeana Forbes, Mem. 68, Cornell Univ. Agr. Exp. Sta. 484. 1924.

A rather large, chunky species with olive-gray primaries and essentially the *hastiana* type of maculation. As noted by Forbes, specimens frequently occur with a blackish basal patch; in others, with obsolescent maculation and paler ground color, the costal triangular patch, with its partial border of black scales, is the most prominent feature of the maculation. In the male

genitalia, the type of socii appears to lead over to the ***latifasciana*** group, although other characters do not bear this out.

Genitalia, Male (Two slides examined). Tegumen broad, without cristae; anal sheath projecting strongly beyond apex of tegumen, unarmed ventrally; socii large, erect, broad at base, tapering apically; sacculus with ventral margin gently sinuate, apical hair-tuft short, broad; aedeagus long, thin, bent strongly; vesica armed with a small rod-like piece of chitin with small apical spine, and three long, fine cornuti, one subapical and the other two close together in central portion.

Genitalia, Female (Two slides examined). Ostium plate narrowly rectangular with short, broad, lateral lobes; ostium broad, slightly concave; chitinous portion of ductus bursae very long, tubular; membranous portion of ductus distended proximally, strongly narrowed beyond the inception of the ductus seminalis; signum an irregularly shaped piece of chitin with strong spines.

Distribution. Quebec, Chelsea, Meach Lake, Kazabazna; Ontario, Ottawa.

Food Plant. Unknown.

Peronea senescens Zell.

Page 326, fig. 2; Page 330, fig. 2.

Teras senescens Zeller, Verh. Zool. Bot. Ges. Wien, XXIV, 431. 1874.

The species has been sunk in the synonymy of *nigrolinea* Rob. from which it is abundantly distinct on genitalic characters. It is slightly smaller in size and narrower winged than *nigrolinea* with less evident raised scale-tufts and a quite variable pattern on forewings. The ground color is light to smoky gray, in some specimens without much maculation except a light sprinkling of black dots; in others the whole median area is strongly suffused with deep smoky; in others again there are more or less distinct oblique sub-basal and median bands of a smokier gray than the ground color; a rarer form shows a black streak from base to apex of wing. The general type of genitalia is that of the *hastiana* group.

Genitalia, Male (Three slides examined). Tegumen broad, without cristae. Anal sheath with a short stout spine with broad base ventro-apically; socii roughly circular; sacculus strongly sinuate, the apical hair-tuft small and subrectangular; aedeagus rather short and stout, vesica armed with a single strong spine (rose thorn) and a minute cornutus.

Genitalia, Female (Two slides examined). Ostium plate narrow, with long, lateral lobes, directed outward and very wide apart, pointed apically; ostium broad, concave, situated at cephalic margin of plate; chitinized portion of ductus bursae very short; membranous portion of ductus rather evenly narrow with slight bulbous enlargement proximally just below which is the inception of the ductus seminalis; bursa contracted somewhat before fundus, with a large stellate signum.

Distribution. British Columbia, Victoria (Saanich District), probably extending into the western United States.

Food Plant. Unknown.

Peronea hastiana Linn.

Page 326, fig. 3.

P. Tortrix hastiana Linn., Syst. Nat. X, 532. 1758.
Acalla hastiana Kennel, Die Palaearct. Tort. 20, Pl. III, figs. 21-43. 1908.
Eclectis hastiana Pierce and Metcalfe, Gen. Brit. Tort. 18, Pl. VII, (♀), Pl. VIII, (♂, as *maccana*). 1922.
Peronea hastiana Sheldon, Entomol. LVI, 75, 1923; *id.*, *op. cit.* LXIII, 148. 1930.
Peronea hastiana Forbes, Mem. 68, Cornell Univ. Agr. Expt. Sta. 485 (*partim*). 1924.
Peronea hastiana Filipjev, Ann. Mus. Zool. Acad. Sci. U.R.S.S., 507, Pl. XLI, fig. 1. 1929; *id.*, *op. cit.* 516, Pl. XXIII, fig. 3, Pl. XXVIII, fig. 6. 1930.

Probably no other species of *Peronea* has been so consistently misidentified in North America as *hastiana*. This is largely due to the known variability of the species in Europe and the consequent attempt on this continent to place a number of good species, which superficially approach *hastiana* forms in color and maculation, under this name; Forbes, for instance, following Dyar's catalogue, places no less than six names under *hastiana*, none of which, according to genitalia, should belong here. In consequence of this confusion it is impossible to recognize the true records of *hastiana* in North America from the misidentifications, and reference to this literature has therefore been omitted.

Even in Europe the species has been subject to misidentification and confusion, and it is only as recently as 1929 that accurate figures of the male and female genitalia have been presented by Filipjev. Several slides made from European material agree with his figures excellently and based on a comparison with these the true *hastiana* has been found to occur fairly commonly in our western provinces. In the specimens examined the variability of pattern is considerable; apart from the more or less typical form, *i.e.*, specimens with deep ruddy brown forewings crossed by an oblique antemedian whitish band, a form with the band of a reddish color is predominant, whilst more unicolorous specimens occur in which the band is almost entirely lost.

Genitalia, Male (10 slides examined). Tegumen with two distinct apical projections (cristae of Filipjev); anal sheath with a small ventro-apical spine; socii moderately large, drooping and roughly triangular; sacculus strongly emarginate at half with rugose edge beyond emargination, apical hair-tuft moderate, rather narrow; aedeagus short, stout, curved, vesica armed with a small, triangular, chitinous plate and four (very occasionally three) fine spines.

Genitalia, Female (Five slides examined). Ostium plate with long, broad, lateral lobes; ostium with raised chitinous lateral flaps; ductus bursae broad, strongly chitinized proximally to a point beyond the termination of the lobes, with a rounded lateral projection on each side; membranous portion of ductus rectangularly broadened proximally, narrowed considerably beyond half; signum small, stellate.

The length and width of the chitinized portion of the ductus appear to be somewhat variable and on the whole the female genitalia offer less reliable characters than do the male organs whereby the species may be differentiated from the two following ones.

Distribution. Manitoba, Aweme; Saskatchewan, Indian Head; Alberta, Edmonton, Red Deer, Fish Creek; British Columbia, Salmon Arm.

Food Plant. *Salix* (in Europe). No food plant records definitely known

Peronea celiana Rob.

Page 326, fig. 4; Page 330, fig. 3.

Teras celiana Robinson, Trans. Am. Ent. Soc. II, 283, Pl. VII, fig. 74. 1869.
Acleris albilineana Kearfott, Can. Ent. XXXIX, 158. 1907.

Robinson's figure of this species is not at all bad. The coloration of the forewings in normal specimens is distinctly red-brown and there is an oblique whitish antemedian band, sprinkled with dark dots, and with rather waved edges. The thorax shows distinct red-brown tufting, which apparently is lacking in *hastiana*. *Albilineana* Kft. of which a Paratype is in the Canadian National Collection, proves to be a form of *celiana*, analogous to one of the European forms of *hastiana*. Both forms (in the dozen or so specimens examined) are remarkably constant and show neither intergrades nor variations.

Genitalia, Male (Four slides examined, one being of *albilineana*). Abundantly distinct from *hastiana*. Cristae of tegumen larger and closer together; ventro-apical armature of anal sheath consists of a transverse ridge of chitin rather than a pointed spine; socii long and narrow, the distal ends being much more drawn out than in *hastiana;* ventral margin of sacculus more sinuate beyond the median emargination than in *hastiana* and with the apical hair-tuft shorter and broader at base; armature of vesica consists of a strong spine situated on a heart-shaped chitinous base and a bundle of short, fine spines, apparently variable in number (6–12).

Genitalia, Female (Two slides examined, one being of *albilineana*). Considerably larger than in the preceding species, but essentially of same type; lateral projections of the chitinous portion of the ductus much larger and the lateral lobes of the ostium plate somewhat more pointed than in *hastiana*.

Distribution. Quebec, Knowlton, Aylmer; Ontario, Ottawa, Biscotasing; Alberta, Edmonton.

Food Plant. *Betula*, according to specimen bred by the writer at Knowlton, Quebec.

Peronea pulverosana Wlk.

Page 326, fig. 5; Page 330, fig. 4.

Teras pulverosana Walker, Cat. Lep. Het. Brit. Mus. XXVIII, 291. 1863.

There is still some doubt as to whether this name is correctly applied; the type male in the British Museum from St. Martin's Falls, Albany river, Ontario, is without abdomen and, in consequence, it has been impossible to examine the genitalia. However, a photograph of this type is before the writer and Mr. W. H. T. Tams of the above institution has kindly picked out and labelled as agreeing with the type one of a number of specimens sent for comparison after a study of the photograph; the determination rests for the present on this specimen.

The species is very close to *hastiana* and would appear, as far as present knowledge goes, to represent it in the eastern portion of Canada. The maculation is essentially as in *hastiana;* in most of the specimens there is less of the ruddy shade in the ground color, and beyond the oblique band the fore-

wing in several of the males appears to be more splotched with a paler gray color, similar to that of the band; two of the males show a distinct dark streak extending from base of wing along the cubital vein to end of cell. The females are smaller and duller in coloration.

Genitalia, Male (Five slides examined). Quite similar to those of *hastiana*, but differing in all specimens examined in the following points; the socii are less drawn out distally and consequently more nearly circular in outline; the aedeagus is slightly longer and narrower and the armature of the vesica consists, besides the triangular piece of chitin, of only *three* spines, somewhat finer and shorter than those of *hastiana;* generally the proximal edge of the excavation of the sacculus forms more nearly a right angle with the basal portion, but this is probably a variable character.

Genitalia, Female (Two slides examined). Quite similar to those of *hastiana* and cannot be satisfactorily differentiated at the present time.

Distribution. New Brunswick, Fredericton (1 ♂); Quebec, Hull (1 ♂), Meach Lake (1 ♀); Manitoba, Aweme (3 ♂, 1 ♀).

Food Plant. Unknown.

Peronea walkerana n. sp.

Page 326, fig. 6; Page 330, fig. 5.

Male

Size of *hastiana*. Head, thorax and forewings a very dark smoky-gray with scarcely a trace of maculation. The oblique antemedian band is very faintly indicated by a few dark scales near costa representing its outer margin and there are scattered blackish scales in apical portion (in one specimen intermingled with stray brown scales). Secondaries pale smoky, slightly speckled with black near apex of wing.

Female

Very similar to male; raised blackish scales on inner and outer margins of the antemedian band better defined than in male, with traces of the deeper costal shading beyond the band usual in members of this group.

Genitalia, Male (Two slides examined). Tegumen with cristae present, but reduced; spine of anal sheath stronger than in *pulverosana;* socii much as in *pulverosana;* sacculus sharply, rectangularly emarginate before half, then almost straight to apical tuft; aedeagus short, stout, vesica armed with an oblong piece of chitin with a raised ridge and *four* spines, considerably *chunkier and shorter* than in *hastiana.*

Genitalia, Female (Three slides examined). Abundantly distinct from *pulverosana.* Lateral lobes of ostium plate shorter and broader; ostium broad, concave, *without lateral flaps;* proximal portion of ductus bursae well chitinized and extending beyond lateral lobes but *without lateral projections;* membranous portion of ductus enlarged proximally, bulb-shaped, containing a large, more or less dumb-bell shaped, lightly chitinized plate; whole ductus shorter than in *pulverosana;* signum stellate.

Holotype ♂, Trenton, Ontario, April 21 (J. Evans); No. 3745 in the Canadian National Collection, Ottawa.

Allotype ♀, Aylmer, Quebec, April 28 (C. H. Curran); in same collection.

Paratypes 1♂, Merivale, Ontario, April 16 (G. S. Walley); 1 ♀, Aylmer, Quebec, April 25 (C. H. Curran); 1 ♀, Trenton, Ontario, June 28 (J. Evans).

The type specimens are evidently hibernated ones and it is probable that in fresh material the maculation will be better defined. The species should not, however, be difficult of recognition, especially in the female sex, after an examination of the genitalia.

Food Plant. Unknown.

Peronea robinsoniana Forbes

Page 326, fig. 7; Page 330, fig. 6.

Teras flavivittana Robinson (*nec* Clemens), Trans. Am. Ent. Soc. II, Pl. VII, fig. 61. 1869.
Peronea robinsoniana (Kft.), Forbes, Mem. 68, Cornell Univ. Agr. Exp. Sta., 487. 1924.
Peronea clemensiana (Kft.), Forbes, *op. cit.* 487. 1924.

The above two names of Kearfott were validated by Forbes in his "Lepidoptera of New York and Neighboring States." The writer has examined, through the courtesy of the American Museum authorities, a series of both forms from the Kearfott collection which he believes may be regarded as co-types and has so marked them. *Robinsoniana* appears to be what Robinson figured under the name *flavivittana* Clem. and *clemensiana* is only a darker form of the same, the forewings being largely suffused with black-brown. Unfortunately neither of the names represents the normal form which is so close in color and maculation to *inana* Rob. as to be almost indistinguishable without a reference to the genitalia. The size is slightly smaller than in *inana* and the coloration of the forewings rather brighter; there is, however, the same whitish basal area defined outwardly by an oblique line and containing a large triangular dark patch on inner margin; the outer half of wing is red-brown with at times slight darker suffusions in costal area. The species is a very variable one, the pale basal area being frequently suffused with the same ruddy-brown found in the apical area; other specimens show a more or less even red-brown shade over the entire wing with white streaks, tending to form a band, along the inner margin, and slight streaking of a similar color below the apex of the wing.

Genitalia, Male (Eight slides examined). Cristae of tegumen and spine of anal sheath much as in *hastiana;* socii narrower and more nearly triangular than in *hastiana*, with the distal portion considerably more produced at both ends; sacculus emarginate at half, edge rugose beyond emargination with a *small, but distinct pointed flap* of chitin; aedeagus short, chunky, vesica armed with a short stout spine (rose-thorn of Pierce) and *five* longer, thinner spines arranged in two groups.

Genitalia, Female (Five slides examined). Ostium plate narrower than in *hastiana;* ostium without the lateral flaps, openly V-shaped; chitinized portion of ductus without lateral projections. Otherwise much as in *hastiana*.

Distribution. Manitoba, Aweme; Alberta, Edmonton; British Columbia, Salmon Arm, Vancouver. Also occurring at Hampton, New Hampshire, and Winchendon, Massachusetts.

Food Plant. Not definitely recorded, possibly *Vaccinium*.

Peronea flavivittana Clem.

Page 326, fig. 8; Page 330, fig. 7.

Peronea flavivittana Clemens, Proc. Ent. Soc. Phil. III, 516. 1864.
Teras perspicuana Robinson, Trans. Am. Ent. Soc. II, 280, Pl. VII, fig. 62. 1869.

The so-called "type" specimen of this species in the collection of the Academy of Natural Sciences, Philadelphia, will have to be rejected as such, according to notes recently made for the writer by Mr. W. J. Brown. This spurious type fits in well with Robinson's figure of the species, Plate VII, fig. 61, but contradicts entirely the original description, the yellow stripe along the inner margin, on which the name was based, being entirely lacking. However, a specimen from New Brighton, Pennsylvania, is before the writer which agrees excellently with Clemens' diagnosis and the identity of the species is based on this specimen.

The typical form is evidently an aberrant one of rather rare occurrence; the more normal form is the one which has been distributed by collectors in Pittsburgh, Pennsylvania, and vicinity under the name *hastiana* and has the forewings of a general blackish-brown color with light brown spotting along costa and outer margin and strong scale-tufting which give the wings a rather roughened appearance. There is considerable variation in the series examined, paler specimens showing a deep blackish basal area and a more or less evident oblique antemedian band varying in color from light creamy to pale brown, the terminal area being purple-brown; the name *perspicuana* Rob. is probably based on an extremely pale specimen of this nature; the locality "Pennsylvania" for the type specimen would certainly point in this direction. Other specimens are more evenly purple-brown with pale streaking along inner margin, quite reminiscent of some of the forms of *robinsoniana*, and others again show heavy black longitudinal streaks through the centre of wing between base and apex.

Genitalia, Male (Seven slides examined). Cristae of tegumen well developed; anal sheath *without* ventro-apical spine; socii smaller and less produced distally than in *hastiana;* sacculus with deep emargination at half, edge strongly rugose beyond; aedeagus *thin, long,* strongly bent, *armed with an apical spine;* vesica with two stout cornuti, situated one above the other.

Genitalia, Female (Five slides examined). Ostium plate with long lateral lobes; ostium rather narrow, concave, with strong, lateral, spiculate flaps; chitinous portion of ductus bursae short, not extending as far as ends of lateral lobes, the following membranous portion somewhat distended to form a long, oval sack, very faintly chitinized and longitudinally streaked; beyond this the ductus is long and narrow with the ductus seminalis situated about midway between the end of the basal sack and the bursa; signum large, stellate.

Distribution. Ontario, Ottawa (this record based on a single female). Other records are Hampton, New Hampshire; Clinton, New York; Pittsburgh and Oak Station, Pennsylvania.

Food Plant. Unknown.

Peronea youngana n. sp.

Page 326, fig. 9; Page 330, fig. 10.

Very evidently allied to the preceding species, but somewhat smaller and paler. Palpi, head, and thorax light gray-brown; primaries light gray-brown with scattered black sprinkling; a strong black dash extends from base of wing along cubital vein to end of cell; a second black dash, starting immediately costad of the end of the first one, continues to apex of wing, both dashes bordered with red-brown scaling; a strong oblique row of raised scales extends across median area of wing and there is a scale tuft on each side of the black basal dash at slightly less than half its length, the lower tuft much the stronger. Secondaries pale smoky, deeper outwardly. Expanse, 18 mm.

In one male paratype the color of primaries is rather even deep gray and in place of the black dashes there is a broad burnt-orange fascia from base half-way to apex; in a female paratype the black dashes are lacking.

Genitalia, Male (Two slides examined). Cristae of tegumen strongly developed; ventro-apical spine of anal sheath *long and spatulate* at tip; socii broad and roughly equilaterally triangular, much larger than in *flavivittana*; sacculus *drawn out to a sharp point* at half, followed by the usual emargination; aedeagus thin, somewhat tapering, armed with a short apical spine; vesica *with an apical bundle of short spines, approximately six to eight in number.*

Genitalia, Female (Two slides examined). Ostium plate with long, broad lobes; ostium rather narrow, strongly convex, with smaller lateral flaps than in *flavivittana;* proximal chitinous portion of ductus bursae short, scarcely extending below lower margin of ostium plate, followed by a bulbous membranous dilation which in turn narrows to form a thin tube; the proximal end of this, almost as far as the inception of the ductus seminalis, is lightly chitinized. *Signum moderate, stellate.*

Holotype ♂, Ottawa, Ontario, June 16, 1905 (C. H. Young); No. 3747 in the Canadian National Collection.

Allotype ♀, same locality and collector, September 25, 1905, in same collection.

Paratypes 1 ♂, 2 ♀, same data, September 3, 16; October 1, 1905.

Food Plant. Unknown.

Peronea britannia Kft.

Page 326, fig. 10; Page 330, fig. 9.

Acleris britannia Kearfott, Can. Ent. XXXVI, 138. 1904.

An easily recognized species with pale orange-yellow forewings with large purple-brown pale-centered costal triangle and slight dark sprinkling or reticulation; the variation in the series examined is very slight. On Vancouver Island the larvae are reported as injurious to leaves of young loganberry canes. According to genitalic characters the species would fall in the *hastiana* group.

Genitalia, Male (One slide examined). Tegumen with distinct cristae; anal sheath with small ventro-apical spine; socii subtriangular, decumbent, produced distally; sacculus with emargination at half; aedeagus rather short, chunky, ***armed with sharp subapical spine***, vesica with two pairs of moderately long cornuti.

Genitalia, Female (One slide examined). Ostium plate narrow, with broad lateral lobes situated wide apart; ostium broad, slightly convex; ductus bursae with proximal section flask-shaped, strongly chitinized to a point beyond ends of lateral lobes; membranous portion of ductus broadened proximally as in *hastiana*, considerably narrowed at half, beyond which is the inception of the ductus seminalis; signum large, stellate.

Distribution. Alberta, Banff; British Columbia, Revelstoke, Kaslo, Salmon Arm, Victoria, Departure Bay.

Food Plants. Loganberry, rose.

Peronea fragariana Kft.

Page 326, fig. 11; Page 330, fig. 8.

Acleris fragariana Kearfott, Can. Ent. XXXVI, 140. 1904.
Peronea fragariana Criddle, 60th Ann. Rep. Ent. Soc. Ont., 25. 1929.

The species has been confused with the European *permutana* Dup. from which it is distinct on genitalic characters; it is to quite an extent a pest on strawberry plants in the western provinces where it is known as the strawberry leaf cutter. The forewings are normally pale orange-yellow in the basal half with reddish-purple suffusion over the outer area and broadly along inner margin to base. The only eastern specimens examined are entirely suffused with deep purple-brown shades on the forewings. On genitalic characters the species shows points of contact with the *inana* group.

Genitalia, Male (One slide examined). Tegumen strongly produced, with slight traces of cristae; anal sheath armed with a blunt ventro-apical spine; socii large, subtriangular, well-produced at both ends; sacculus with lower margin straight, except for a small semicircular projection with rugose edge at half; aedeagus moderately long and narrow; vesica with a strong curved apical spine and three minute cornuti.

Genitalia, Female (One slide examined). Ostium plate large with convex caudal margin and broad lateral lobes, rather close together; ostium near cephalic edge of plate, straight, broad; ductus bursae with the proximal chitinous portion long, tubular, extending well beyond apex of lateral lobes; membranous portion broadened proximally much as in *hastiana*, then considerably narrowed with inception of ductus seminalis near distal end; bursa large with large stellate signum.

Distribution. New Brunswick, Fredericton; Manitoba, Aweme, Souris; Saskatchewan, Saskatoon.

Food Plant. Strawberry and probably other allied plants.

Peronea inana Rob.

Page 327, fig. 1; Page 331, fig. 1.

Teras inana Robinson, Trans. Am. Ent. Soc. II, 281, Pl. VII, fig. 65. 1869.

The determination of this species is based on a specimen compared by the writer in 1925 with a series in the United States National Museum under this name, and by a recent examination of a specimen in the American Museum Collection labelled "Homotype" by Kearfott. The small series of 2 ♂, 4 ♀, in the Canadian National Collection shows no sign of variation and agrees quite well with Robinson's figure, but is disconcertingly close in general appearance to what has been already discussed as the normal form of *robinsoniana*. As far as can be told, *inana* is a rather larger species, with less prominent pure white scaling on rear portion of thorax and with the basal area of forewing rather evenly dull gray-brown without the contrasting white scaling or the obscuring blackish shading frequently found in *robinsoniana* forms. Until, however, one knows the species well, the genitalia form the only safe means of determination.

Genitalia, Male (Two slides examined). Tegumen *without* cristae; no armature to anal sheath; socii long, well-produced distally, suboval in shape; sacculus *without* emargination, but with *small semicircular projection* beyond half; aedeagus short, moderately stout, vesica armed with a strong, short, subapical spine (rose-thorn) and a bundle of about a dozen small cornuti.

Genitalia, Female (Four slides examined). Ostium plate rather narrow from front to rear, lateral lobes broad, long and rather wide apart; ostium broad, concave; ductus bursae broad proximally and strongly chitinized for a considerable length to a point beyond ends of lateral lobes, expanding somewhat laterally at termen; membranous portion of ductus as broad as chitinous portion at commencement, but narrowing rapidly, forming a long narrow tube beyond the inception of the ductus seminalis. Signum large, roughly circular, scobinate.

Distribution. Ontario, Ottawa; Alberta, Edmonton.

Food Plant. Unknown.

Peronea busckana n. sp.

Page 327, fig. 2; Page 331, fig. 3.

Female

Palpi, head and thorax purple-gray, the latter shaded on patagia and tuft with brown and crossed by a median black line. Forewings light violet-gray shaded with deeper purple-gray. At base of wing a small black-brown patch, shaded slightly with light brown scaling; just beyond this in the fold a smaller dark patch with brownish scaling and a dark scale-tuft, this patch enclosed in a broad oblique band of the violet-gray ground color which appears broader at inner margin than at costa and is defined outwardly in costal half and on inner margin by an oblique row of 4 or 5 minute, blackish, slightly raised dots, faintly and partially surrounded with light brown scaling. Beyond this band, in costal half of wing a broadly triangular patch, extend-

ing nearly to apex of wing, is obscurely indicated by darker, purple-gray suffusion. The terminal area of wing is violet-gray with a few scattered black dots. Fringes light violet-gray with some darker scales at base. Secondaries very pale smoky, somewhat darker apically.

Male

Rather paler than the female in color with the basal patch and costal triangle less obvious; the slightly raised dark dots partially defining the outer edge (and at times the inner edge) of the antemedian band are, however, quite prominent and there is a further more or less incomplete line of similar dark dots from costa at about half to inner margin before anal angle, strongly excurved opposite cell. These dots at times give a very characteristic appearance to the wing. Expanse, ♂, 20–22 mm., ♀, 20 mm.

Genitalia, Male (One slide examined). Cristae of tegumen large and sharply pointed; anal sheath with moderate ventro-apical spine; socii small, not produced distally; claspers bent inward in apical half; sacculus with straight basal portion, then with strong emargination beyond half, roughly rectangular at anal angle with strong terminal hair-tuft; aedeagus rather chunky; vesica armed with a large spindle-shaped piece of chitin and a smaller spine without base.

Genitalia, Female (Three slides examined). Very characteristic and quite distinct from all others in the group. Ostium plate with the lobes broad, but rather short, slightly bent outward at extremity; ostium broad, convex, forming a hood-like, heavily chitinized plate, the chitinization extending downward along the inner edges of the lateral lobes; the proximal, strongly chitinized portion of the ductus bursae with parallel sides, extending well beyond the ends of lobes; membranous portion rather short, narrowed towards bursa. Signum roughly circular, scobinate.

Holotype ♀, Mer Bleue, near Ottawa, Ontario, September 18, 1902 (C. H. Young); No. 3748 in the Canadian National Collection, Ottawa.

Allotype ♂, Hampton, New Hampshire, October 10, 1907 (S. A. Shaw); in Collection of the American Museum of Natural History, New York.

Paratypes 1 ♀, Aweme, Manitoba, October 3, 1923 (N. Criddle); in Canadian National Collection; 6 ♂, 2 ♀, Hampton, New Hampshire, October 10, 16, 26, November 20, December 3, April 30, May 13; 4 ♂, 11 ♀, Winchendon, Massachusetts, October 12, 14, 20, 26, 28, 31, April 17, May 9, 25, in Collection of the American Museum of Natural History and Canadian National Collection.

A long series from the Kearfott Collection, loaned by the American Museum of Natural History, was very useful in augmenting the scanty number of specimens available for study in our own collections. There is very little variation in either color or pattern in the specimens examined.

This species somewhat resembles *similis* Filipjev in type of genitalia, especially in the female sex.

Food Plant. Unknown, but possibly *Vaccinium* or an allied plant.

Peronea maculidorsana Clem.

Page 327, fig. 10; Page 331, fig. 2.

Cnephasia ? maculidorsana Clemens, Proc. Ent. Soc. Phil. III, 516. 1864.
Teras maculidorsana Robinson, Trans. Am. Ent. Soc. II, 281, Pl. VII, fig. 64. 1869.
Peronea hypericana Ely, Proc. Ent. Soc. Wash., XII, 68. 1910.

The species has probably been confused with both *inana* and *busckana;* it is a much grayer species than the former and Robinson's figures emphasize this difference quite clearly. From *busckana* it differs in having a dark lunate patch above inner margin near base of wing, which is entirely pale gray; this patch is much more clearly defined in the females than in the males which are frequently without much maculation or brown suffusion and superficially resemble closely *busckana*, the dark raised dots (when present) being similarly placed in both species; otherwise, there does not appear to be much variation. From the description and from memory of the type in the United States National Museum, *hypericana* Ely would appear to fall as a synonym.

Genitalia, Male (Two slides examined). Small for the size of the insect. Tegumen *without* cristae and anal sheath without armature; socii rather small, roughly triangular; sacculus margin almost straight with the exception of a small semicircular chitinous flap, projecting beyond half; aedeagus moderately stout, *armed with a short apical spine;* vesica with a bundle of small cornuti, variable in number (8–12 in specimens examined).

Genitalia, Female (Two slides examined). Ostium plate with *only an indication* of lateral lobes, ostium broad and close to lower margin of plate (as drawn); chitinized portion of ductus bursae short and roughly subrectangular; membranous portion broad, slightly enlarged proximally, bursa small with signum represented by a long, narrow, scobinate strip of chitin.

Distribution. Quebec, Aylmer, Kazubazua; Ontario, Constance Bay, Mer Bleue (both localities in the Ottawa region). Also occurs at Hampton, New Hampshire.

Food Plant. Hypericum (according to Ely). In the Ottawa region the normal food plant would appear to be *Vaccinium.*

Peronea scabrana D. & S.

Page 327, fig. 3; Page 331, fig. 4.

Tortrix scabrana Denis and Schiffermuller, Syst. Verz. 130. 1776.
Peronea scabrana Filipjev, Ann. Mus. Zool. Acad. Sci. U.R.S.S. 507, Pl. XLI, figs. 2, 4. 1929.

This species, which has only recently been removed from the synonymy of *hastiana* by Filipjev, occurs also in North America. A single male in the Canadian National Collection from Calgary (*ex* Coll. Wolley-Dod) has been recently supplemented by several specimens secured by Mr. K. Bowman at Nordegg, Alberta.

As stated by Filipjev, the species is longer and narrower-winged than *hastiana* and appears to be as variable in color and maculation as this species. Our Calgary specimen has pale fawn-colored primaries with a blackish basal patch, the outer edge of which is acutely angled outwardly in middle of wing; the other specimens, although with similar genitalia, are of a more or less even deep gray color with very obscure maculation.

Genitalia, Male (Two slides examined). Tegumen broad and without cristae. Armature of anal sheath a long acute spine (longer than in European specimens); socii club-shaped, rugose rather than hairy; margin of sacculus gently sinuate; aedeagus long, narrow, with short terminal spine; vesica armed with a short, pointed piece of chitin and three cornuti.

Genitalia, Female. Ostium plate with long pointed lobes; ostium rather narrow, slightly concave; chitinized portion of ductus bursae short, subrectangular; membranous portion of ductus strongly enlarged in proximal half, much narrowed after inception of the ductus seminalis. Signum moderate, scobinate.

Distribution. Alberta, Calgary, Nordegg, Edmonton.

Food Plant. Unknown.

Peronea lipsiana D. & S.

Page 327, fig. 9; Page 331, fig. 6.

Tortrix lipsiana Denis and Schiffermuller, Syst. Verz. 129. 1776.
Acleris lipsiana Kennel, Palaearct. Tort. 90, Pl. V, figs. 28-30. 1909.
Peronea lipsiana Pierce and Metcalfe, Gen. Brit. Tort. 22, Pl. IX (?). 1922.
Peronea lipsiana Filipjev, Ann. Mus. Zool. Acad. Sci. U.R.S.S., 516, Pl. XXIV, fig. 1; Pl. XXIX, fig. 4. 1930.
Peronea lipsiana Sheldon, Entomol. LXIII, 273. 1930.

The species has been heretofore unrecognized in North America and probably confused with *hudsoniana* Wlk. (*brewsteriana* Rob.). A small series from the western provinces, however, not only agrees excellently in maculation with Kennel's figures, but in genitalic characters matches exactly Filipjev's figures of these organs; Pierce's figures under this name show some discrepancies and only doubtfully represent the species.

The primaries of *lipsiana* are practically smooth, rather even dark gray with a slight fawn-colored tinge and sprinkled lightly with black dots; this sprinkling is particularly evident in the females, which are generally paler gray and considerably smaller in size. In another equally common form the ground color is ruddier with the costal triangle and a patch on inner margin near base indicated by smoky shading.

Genitalia, Male (Three slides examined). Tegumen narrow apically, without cristae; anal sheath unarmed; socii on a long, narrow, rod-like base, small, hemispherical, with short hairs; margin of sacculus gently arched beyond half, rugose; apical hair-tuft strong, pointed; aedeagus thin, vesica armed with a small triangular piece of chitin and a bundle of numerous long thin cornuti, about 15–18 in number.

Genitalia, Female (Three slides examined). Ostium plate with long, broad lobes, bent slightly inward apically; ostium broad, sinuate; ductus bursae with the chitinized portion unusually long and narrow, extending far beyond the ends of the lateral lobes; proximal end of membranous portion of ductus slightly enlarged and containing a chitinized plate below which is the inception of the ductus seminalis; remainder of ductus bursae short and rather broad; signum large, considerably longer than broad, well-spined.

Distribution. Manitoba, Aweme; Alberta, Edmonton.

Food Plants. ***Vaccinium, Myrica*** (in Europe).

Peronea hudsoniana Wlk.

Page 327, fig. 8; Page 331, fig. 9.

Teras hudsoniana Walker, Cat. Lep. Het. Brit. Mus. XXVIII, 290, (♂). 1863.
Sciaphila implexana Walker, Cat. Lep. Het. Brit. Mus. XXVIII, 338, (♀). 1863; Walsingham, Ill. Lep. Het. Brit. Mus. IV, 3, Pl. LXI, fig. 7 (as *pulverosana* Wlk.). 1879.
Teras brewsteriana Robinson, Trans. Am. Ent. Soc. II, 283, Pl. VII, fig. 73. 1869.

The correct identity of the various specific names involved in the above synonymy has been difficult to determine. Walsingham's and Robinson's descriptions and figures distinctly indicated the sinking of *brewsteriana* to *implexana* and comparisons of specimens kindly made by Mr. W. H. T. Tams in the British Museum with the type female of *implexana*, and by Dr. E. T. Cresson, Jr., in the Academy of Natural Sciences, Philadelphia, with the type of *brewsteriana* confirmed this opinion. With regard to *hudsoniana*, however, Mr. Tams was unwilling to make a definite decision, the abdomen of the type being lost and the maculation of the specimen showing a marked similarity to that of several allied species. After a careful study of a photograph of the type, the writer has rightly or wrongly concluded that *hudsoniana* most probably was based on the male sex of *implexana;* in the fairly large series of the species available for study the males are consistently much larger than the females, more evenly deep gray in the coloration of the primaries, and with the brown shading beyond the discal dash, so characteristic for the female, much reduced or entirely lacking. There is little variation in our series except that occasionally in the male the forewings are irregularly splotched with small, dark patches.

The genitalia in both sexes are remarkably characteristic.

Genitalia, Male (Four slides examined). Tegumen without cristae; anal sheath *bifid* at apex, armed with a strong ventral spine; socii long, narrow, drawn to a point distally; sacculus strongly emarginate at half, abruptly and rectangularly produced costad at apex, which is raised above the plane of the clasper and tufted with hairs; aedeagus long, narrow, armed with a short terminal spine; vesica with three terminal cornuti, one of which is only half the length of the others.

Genitalia, Female (Five slides examined). Ostium plate *entirely without* lobes, ostium nearly straight, occupying the entire width of the cephalic (lower, in figure) margin of the plate; ductus bursae as broad as the ostium plate at its inception, strongly chitinized for a considerable length and asymmetrically narrowing; a large oval bulge from the under-surface occupies the adjacent portions of both the plate and the ductus; beyond the chitinized portion the ductus forms an irregularly shaped sack at the lower end of which the ductus seminalis has its inception; remainder of ductus narrow; bursa small with a minute stellate signum.

Distribution. Ontario, Ottawa; Manitoba, Aweme; Saskatchewan, Indian Head; Alberta, Fish Creek, Edmonton, Red Deer, Nordegg. Specimens also examined from Hampton, New Hampshire; Winchendon, Massachusetts; and Montclair, New Jersey.

Food Plant. Unknown.

Peronea bowmanana n. sp.

Page 327, fig. 4; Page 331, fig. 5.

Male

Palpi deep purple-gray with slight ruddy scaling above; head and thorax deep purple-gray, latter with a few burnt-sienna scales on the tuft. Primaries almost unicolorous deep purple-gray (at times without purple shade) with a slight admixture of burnt-sienna scaling and a small ruddy shade at base of wing. A prominent tuft of creamy white raised scales (very easily rubbed off) surrounding two minute black scale-dots in the cell. Secondaries semi-hyaline, whitish, slightly smoky apically.

Female

Smaller than male; ruddy shades on thoracic tuft and at base of forewing more prominent; white discal scale-tuft very strong in unrubbed specimens. Secondaries somewhat smokier in color. Expanse, ♂, 22 mm., ♀, 18–20 mm.

Genitalia, Male (Four slides examined). Tegumen without cristae, broad, expanded laterally at bases of socii; anal sheath without armature; socii long, ribbon-like, pointed apically, shortly hairy in apical section; claspers short, chunky; sacculus slightly emarginate at half, then strongly rounded with short, broad apical tuft; apex of valvula thickened, rugose; aedeagus rather narrow, strongly bent, vesica armed with a single long stout spine.

Genitalia, Female (Two slides examined). Ostium plate with long, narrow lateral lobes, slightly directed inwardly; ostium broad, sinuate; chitinized portion of ductus bursae short, rectangular; membranous portion of ductus with a slight proximal enlargement, containing a faintly chitinized plate, this section slightly narrowed distally and followed by a further expansion to a short broad tube leading into the small bursa. Signum very minute, almost obsolete.

Holotype ♂, Edmonton, Alberta, October 1, 1932 (K. Bowman); No. 3750 in the Canadian National Collection, through the courtesy of the collector.

Allotype ♀, Aweme, Manitoba, October 3, 1923 (N. Criddle); in same collection.

Paratypes 1 ♂, 6 ♀, Edmonton, Alberta, March 28, April 22–29 (K. Bowman); in Canadian National Collection and collection of K. Bowman; 2 ♂, Aweme, Manitoba, October 2, May 13 (N. Criddle); in Canadian National Collection; 4 ♂, 1 ♀, Aweme, Manitoba, April 4, 8, 14 (Criddle) (ex Coll. Kearfott) in Collection of the American Museum of Natural History; 1 ♂, 2 ♀, Salmon Arm, British Columbia, October 17, March 13, May 7 (W. R. Buckell); in Canadian National Collection and collection of W. R. Buckell.

Food Plant. Unknown.

Peronea minuta Rob.

Page 327, fig. 7; Page 331, fig. 8.

Tortrix minuta Robinson, Trans. Am. Ent. Soc. II, 276, Pl. VI, fig. 49. 1869.
Tortrix vacciniivorana Packard, 17th Ann. Rep. Sec. Mass. Brd. Agr. 241. 1870.
Tortrix malivorana Le Baron, 1st Ann. Rep. Ins. Ill. 20. 1870; Riley, 4th Ann. Rep. State Entom. Mo. 47. 1872.
Tortrix cinderella Riley, 4th Ann. Rep. State Entom. Mo. 46. 1872; *id.* Bull. 6, U.S. Entom. Comm. 82. 1881.
Teras variolana Zeller, Verhandl. zool. bot. Ges. Wien, XXV, 212. 1875.

This species is the well known pest on cranberry plants and the larva is known to economic entomologists as the yellow-headed fire worm. In the middle western States it was found destructive to apple trees and described as a new species, but Riley later concluded that apple and cranberry-feeders were identical and his views have been generally followed and are here accepted. The adults occur in two main color forms; the typical one with orange-yellow forewings, faintly reticulate with shining purplish scaling, occurs mostly in the spring and summer generations; the fall generation (*cinderella* Riley) which hibernates has the forewings deep smoky gray. In Canada the species is apparently not very common and is only found in bogs where blueberries abound. The characteristic genitalia show no very close relationship to any of the *Peronea* groups, but possibly link up best with the *hudsoniana-bowmanana* group.

Genitalia, Male (Six slides examined). Very small. Tegumen broad apically, especially at bases of socii; anal sheath weak, unarmed; socii long, narrow, bluntly pointed, with short scattered hair apically; claspers short; broad, sacculus strongly excavated beyond base, bent upward toward costa apically with raised hair-tuft; aedeagus large, broad; vesica armed with a chitinous plate with raised ridge and an apical cluster of 8 to 10 small cornuti.

Genitalia, Female. Ostium plate with very short lateral lobes; ostium broad, situated on cephalic margin of plate; chitinized proximal portion of ductus bursae long, broad, finely scobinate; membranous portion of ductus enlarged to an elongated sack shortly beyond base, narrowing at about half, where the inception of the ductus seminalis is situated; signum broadly oval, spined.

Distribution. Ontario, Ottawa (Mer Bleue); Manitoba, Aweme; Alberta, Fish Creek, Nordegg.

Food Plants. Apple, cranberry.

Peronea logiana Linn.

Page 327, fig. 5; Page 331, fig. 7.

P. Tortrix logiana Linnaeus, Faun. Suec. (Ed. II), 348. 1761.
Phalaena niveana Fabricius, Ent. Syst. III (2), 267. 1794.
Teras placidana Robinson, Trans. Am. Ent. Soc. II, 282, Pl. VII, fig. 68. 1869.
Teras trisignana Robinson, Trans. Am. Ent. Soc. II, 282, Pl. VII, fig. 69. 1869.
Acalla niveana Kennel, Palaearct. Tort. 87, Pl. V, figs. 19, 20. 1909.
Peronea niveana Pierce and Metcalfe, Gen. Brit. Tort. 20, Pl. VIII. 1922.
Peronea trisignana Forbes, Mem. 68, Cornell Univ. Agr. Exp. Sta. 483. 1924.
Oxigrapha logiana Sheldon, Entomol. LXIII, 195. 1930; *id., op. cit.* LXIV, 101. 1931.
Peronea niveana Filipjev, Ann. Mus. Zool. Acad. Sci. U.R.S.S. 522, Pl. XXV, figs. 7, 8, Pl. XXIX, fig. 1. 1930.

The involved synonymy of this species in Europe has been satisfactorily cleared up by Sheldon; there seems no doubt that Robinson's two names represent North American forms of this same species, *placidana* falling to the

type form and ***trisignana*** taking priority (if a name be desired) over ***tripunctana*** Sheldon (*op. cit.* 103). Both Pierce and Filipjev state that in the male genitalia the vesica is armed with *three* spines; in the only European specimen examined *four* spines were present, which agrees with the number found in North American specimens.

Genitalia, Male (Three slides examined). Tegumen rather narrow, produced; anal sheath without armature, the inner, pointed ends being connected by a chitinous membrane with the tegumen to form a hood-like structure; claspers broad, chunky, sacculus slightly sinuate, terminating in a knob-like tuft of hair; aedeagus broad, long, vesica armed with an apical, shorter and a basal, longer pair of strong cornuti, with a ridged chitinous plate between.

Genitalia, Female. Ostium plate with long pointed lateral lobes; ostium broadly V-shaped; chitinous portion of ductus bursae long, broad, extending well beyond ends of lateral lobes; basal half of membranous portion of ductus expanded into an oblong sack beyond which is the inception of the ductus seminalis; signum large, stellate.

Distribution. Quebec, Meach Lake; Ontario, Ottawa, Biscotasing; Alberta, Edmonton; British Columbia, Agassiz.

Food Plant. Birch.

Peronea nivisellana Wlshm.

Page 327, fig. 6; Page 332, fig. 1.

Teras nivisellana Walsingham, Ill. Lep. Het. Brit. Mus. IV, 2, Pl. LXI, fig. 3. 1879.
Peronea nivisellana Barnes and Busck, Cont. N. Hist. Lep. N. Am. IV (3), Pl. XXXII, fig. 9. 1920; Forbes, Mem. 68, Cornell Univ. Agr. Exp. Sta., 484. 1924.

A striking species with the dark head contrasting with the white thorax, the mottled whitish basal half of forewing and the dark purple-black costal patch and terminal suffusion, relieved inwardly by paler brown shades; the scale-tufts below the apex of the costal triangle are unusually prominent. There is little variation in the series under examination.

Genitalia, Male (One slide examined). Tegumen broad apically; anal sheath armed with a sharp ventro-apical spine; socii large, oval, projecting beyond lateral edge of tegumen; claspers narrow, bent upward apically, lower margin of sacculus gently sinuate, terminating in a small, slightly raised hair-tuft; aedeagus thin, armed with a short apical spine, vesica with one long, apical cornutus and another very minute one.

Genitalia, Female (One slide examined). Ostium plate with long, thin, straight, lateral lobes; ostium rather narrow, V-shaped, situated slightly above cephalic margin of plate; ductus bursae entirely membranous, somewhat expanded in proximal third, beyond which is the inception of the ductus seminalis; bursa large, with a long, band-like, spined signum.

Distribution. Quebec, Meach Lake; Ontario, Ottawa, Rostrevor, Biscotasing; Manitoba, Aweme; Alberta, Edmonton; British Columbia, Departure Bay.

Food Plant. Crataegus (*teste* Forbes).

In the remainder of the species under consideration, which belong in what may be termed the *ferrugana* group, the male genitalia show the lateral portions of the tegumen united by a chitinous membrane to the inner apices of the anal sheath, forming a hood-like structure. This will be simply referred to in future as "tegumen, hood-like." The species are all quite similar in general genitalic characters and are separated on the finer details, such as shape of socii, spining of vesica, etc.

Peronea cervinana Fern.

Page 328, fig. 1; Page 332, fig. 2.

Teras cervinana Fernald, Trans. Am. Ent. Soc. X, 65. 1882.
Teras americana Fernald, Trans. Am. Ent. Soc. X, 66. 1882.
Peronea cervinana Barnes and Busck, Cont. N. Hist. Lep. N. Am. IV (3), Pl. XXXII, fig. 7. 1920.
Peronea cervinana Forbes, Mem. 68, Cornell Univ. Agr. Exp. Sta. 483. 1924.

The typical form is of a rather deep orange-brown color on forewing, at times finely reticulate with brown; the costal triangle is dark brown enclosing a striking white rectangular spot based on costa; in the form *americana* the ground color of the forewings is paler, more fawn-colored and the included white spot in the dark costal triangle is replaced by one of the same general color as the main portion of the wing. Another very marked form, of which only a single specimen has been examined, has the thorax largely whitish; in the forewing the basal third is white and the remainder of the wing a deep purple-brown, forming a very striking contrast.

Genitalia, Male (Five slides examined). Tegumen hood-like; anal sheath armed with a strong ventro-apical hood-like projection, socii erect, narrowly oval; claspers broad, chunky, the valvula projecting only slightly beyond apex of costa; margin of sacculus almost straight with slight chitinous flap beyond half and narrow apical hair-tuft; aedeagus moderate, strongly bent and slightly tapering with stout subapical spine, vesica with *two* strong cornuti and a shorter apical one.

Genitalia, Female (Two slides examined). Ostium plate with rather short lateral lobes; ostium broad, semicircular; ductus bursae shortly chitinized at proximal end, following which is a small, bulbous, membranous portion, containing a chitinous plate and narrowing distally; this is followed by a large chitinized sack with narrow band of fine spicules along one side, beyond which the ductus is narrowly tubular, membranous, and with the inception of the ductus seminalis just distad of chitinous sack; signum large, stellate.

Distribution. Quebec, Meach Lake, Kazabazua; Ontario, Ottawa; Alberta, Edmonton.

Food Plant. Unknown.

Peronea subnivana Wlk.

Page 328, fig. 2; Page 332, fig. 3.

Penthina subnivana Walker, Cat. Lep. Het. Brit. Mus. XXVIII, 376. 1863.
Teras deflectana Robinson, Trans. Am. Ent. Soc. II, 283, Pl. VII, fig. 71. 1869.
Teras peculiana Zeller, Verhandl. zool. bot. Ges. Wien, XXV, 210, Pl. VIII, fig. 1, 1875.
Teras subnivana Walsingham, Ill. Lep. Het. Brit. Mus. IV, 1, Pl. LXI, fig. 2. 1879.
Peronea subnivana Forbes, Mem. 68, Cornell Univ. Agr. Exp. Sta. 483. 1924.

A pale whitish or fawn-colored species with the forewings at times distinctly reticulate in light brown; the dark brown costal triangle may be solid or broken by a pale central patch. The costa of forewing is strongly concave (more so in the female sex than in the male) beyond half, and this feature should at once separate it from the allied *cervinana* and its forms.

Genitalia, Male (One slide examined). Very similar to *cervinana;* socii somewhat broader; clasper longer and narrower with the apex of valvula scarcely projecting and broadly rounded; sacculus margin rather more sinuate and without the small chitinous flap at half; aedeagus *much longer and narrower*, with smaller apical spine; armature of vesica consists of *one* long, stout spine and two short, but fairly stout ones.

Genitalia, Female. Ostium plate with lateral lobes slightly longer, narrower and more pointed than in *cervinana;* ostium slightly broader; in the ductus bursae the neck of the chitinized sack is shorter and broader, the sack itself less strongly chitinized and the spiculate band contains fewer spicules.

Distribution. Quebec, Chelsea, Meach Lake; Ontario, Ottawa.

Food Plant. *Vernonia* (*teste* Forbes).

Peronea braunana n. sp.

Page 328, fig. 3; Page 332, fig. 4.

Peronea ferrugana Barnes and Busck (*nec* D. & S.), Con . N. Hist. Lep. N. Am. IV (3), Pl. XXXII, fig. 2. 1920.

Palpi and head smoky brown with an admixture of ochreous; thorax largely red-brown; primaries rather deep red-brown or purple-brown with a slight shading of gray scaling along inner margin; a strong purple-black costal triangle, broken somewhat on costa by a small patch of the ground-color; a faint line of white scaling borders the basal edge of this patch and there are a few slightly raised white and black scales scattered along its inner margin as well as a broken oblique line of similar scales directed from its outer edge toward outer margin of wing above anal angle; a moderately large tuft of black scales in fold near base. Fringes concolorous. Secondaries pale smoky, deeper outwardly. Expanse 15 mm.

Genitalia, Male (Three slides examined). Very similar to those of *cervinana.* Tegumen hood-like; anal sheath produced strongly beyond apex of tegumen; armed with a strong ventro-apical projection; socii upright, *narrower and more pointed* than in *cervinana;* claspers somewhat narrower than in *cervinana* with apex of valvula projecting further beyond costa; sacculus feebly emarginate with somewhat broader chitinous flap and a shorter apical hair-tuft; aedeagus thinner and longer than in *cervinana* armed with a shorter subapical spine, vesica with a single apical and a pair of median cornuti, all of equal length, and slightly less stout than the larger ones of *cervinana.*

Genitalia, Female (Two slides examined). Ostium plate with long, thin, parallel, lateral lobes; ostium much as in *cervinana*, broad, semicircular; basal section of ductus bursae similar to that of *cervinana* with short chitinized portion, followed by a bulbous expansion, containing a chitinized plate; the following sack-like expansion is, however, *entirely membranous;* signum moderately large, stellate.

Holotype ♂, Biscotasing, Ontario, September 9, 1931 (K. Schedl); No. 3751 in the Canadian National Collection, Ottawa.

Allotype ♀, Meach Lake, Quebec, September 29, 1904 (C. H. Young).

Paratypes 3 ♂, same data as holotype, September 12; 1 ♂, same data as allotype, September 23; 1 ♂, Ottawa Golf Club, Quebec, September 14 (F. P. Ide).

Further specimens have been studied from Kazubazua, Quebec; Algonquin Park, Ontario; Salmon Arm, and Alta Lake, British Columbia, the latter a striking form with the basal half of forewing white, as in a very similar form mentioned under *cervinana*.

The species has been generally misidentified as *ferrugana* or *semiannula* but, as is shown later, is distinct from either of these species.

Food Plant. Probably *Betula*.

Peronea kearfottana n. sp.

Page 328, fig. 4; Page 332, fig. 5.

A small species with pale orange-yellow forewings, resembling considerably *minuta* Rob. Head and thorax light yellow-brown; primaries almost unicolorous orange-yellow with faint traces of reticulation and a general shiny appearance; secondaries light smoky with paler fringes. Expanse 15 mm.

In the allotype female and some paratype specimens there is a trace of a smoky oblique shade from costa at half to end of cell and in one specimen (hibernated) the forewing is suffused and reticulate with smoky over a pale creamy ground-color.

Genitalia, Male (Three slides examined). Tegumen hood-like; anal sheath armed with a strong ventro-apical hood-like projection; socii long, narrow, upright; ventral margin of sacculus gently sinuate with small, oval, terminal hair-tuft; aedeagus rather short, stout, bent; vesica with one or two small apical cornuti and a centrally situated pair, one of which is longer and stouter than the other.

Genitalia, Female (One slide examined). Ostium plate with short, pointed lateral lobes; ostium rather broad, semicircular; ductus bursae shortly membranous proximally, followed by an expansion into a large irregularly shaped sack, the neck of which is lightly chitinized, then strongly narrowed before the bursa, which is small, circular and with a small stellate signum.

Holotype ♂, Bobcaygeon, Ontario, June 20, 1932 (reared from *Myrica*) (J. McDunnough); No. 3746 in the Canadian National Collection, Ottawa.

Allotype ♀, same data, June 22, 1931.

Paratypes 2 ♂, Kazubazua, Quebec, July 19, 20 (F. P. Ide); 1 ♂, Biscotasing, Ontario, May 15 (K. Schedl); 2 ♀, Bobcaygeon, Ontario, June 21, July 10 (J. McDunnough); 1 ♀, Ottawa, Ontario, July 3 (C. H. Young).

Food Plant. *Myrica.*

Peronea simpliciana Wlshm.

Page 328, fig. 5; Page 332, fig. 6.

Teras simpliciana Walsingham, Ill. Lep. Het. Brit. Mus. IV, 2, Pl. LXI, fig. 4. 1879.

The identification of this species is based on the excellent agreement of a small series in the Canadian National Collection with the original description and colored figure.

The size is small and the color of forewings whitish with a creamy or olivaceous tinge; there is a strong, blackish, flatly triangular blotch on costa at half which may or may not contain a pale centre, a black dot in the fold at one-third and a faint curved line of leaden scaling from the outer edge of costal triangle to outer margin above anal angle.

Genitalia, Male (Two slides examined). Tegumen hood-like; anal sheath with strong, narrow, truncate projection ventrally; socii upright, rather narrow and blunt apically; claspers moderately wide, sacculus gently sinuate with small, narrowly oval, apical hair-tuft; aedeagus narrow, apex bent at right angles, enlarged, *funnel-shaped;* vesica armed with two minute cornuti, one situated subapically, the other centrally.

Genitalia, Female (One slide examined). Ostium plate broad, lateral lobes incurved and sharply pointed; ostium broad, situated on cephalic margin of plate, ductus bursae with an enlarged, *bowl-shaped,* chitinized portion proximally followed by a long, thin membranous tube with the inception of the ductus seminalis at two-thirds. Signum stellate.

Distribution. Manitoba, Aweme.

Food Plant. Unknown.

Peronea forbesana n. sp.

Page 328, fig. 6; Page 332, fig. 7.

Rather similar to *simpliciana* Wlshm., but larger and the forewings more variegated in appearance, due to the greater prominence of leaden-colored scaling.

Palpi, head and thorax light creamy-white, the latter shaded partially with olive-gray. Primaries with costal margin shaped as in *cervinana* Fern., creamy-white, shaded and suffused with olive-gray and with a prominent sharply defined, purplish-black, broadly triangular costal patch with slight admixture of brown scales, extending from costa at two-fifths to costa at four-fifths; basal area lightly and irregularly shaded with pale olive-gray, the outer edge of this area containing two minute black spots on cubital vein and in the fold; a slight shading of olive-gray on inner margin at half and a broad irregularly oblique band of olive-gray or leaden-colored scaling

from apex of costal patch to anal angle; a small, oval, olive-gray subapical patch and slight reticulations of similar color along outer margin; fringes olive-gray, paler outwardly. Scale-tufting not prominent, when present consisting of a small pale tuft below apex of costal patch in cell and a similar one in the fold at one-third. Expanse 15 to 17 mm.

Genitalia, Male (Four slides examined). Tegumen hood-like; anal sheath with the ventro-apical projection very weak; socii upright, long, slightly more pointed apically than in *simpliciana;* claspers broad, chunky, sacculus very slightly sinuate with narrowly oval apical hair-tuft; aedeagus rather long, thin, vesica armed with a single small apical cornutus, a small triangular piece of chitin with short spine (rose-thorn) and two centrally situated cornuti, slightly larger than the apical one.

Genitalia, Female (Two slides examined). Ostium plate with well-developed lobes, narrow and slightly divergent; ostium small, semicircular; ductus bursae *membranous and moderately broad proximally* to a point slightly beyond apices of lateral lobes, then somewhat constricted to form the neck of a long sack-like enlargement, this neck being lightly chitinized, and the membranous sack containing a narrow band of fine spicules; terminal portion of ductus very narrow. Signum stellate.

Holotype ♂, Bobcaygeon, Ontario, July 1, 1932 (bred from *Cornus*) (J. McDunnough); No. 3749 in the Canadian National Collection, Ottawa.

Allotype ♀, Fredericton, New Brunswick, May 1, 1928 (R. P. Gorham).

Paratypes 1 ♂, same data as holotype, July 6; 1 ♂, Meach Lake, Que., September 25, 1902 (C. H. Young); 1 ♂, Ottawa, Ontario, September 22, 1905 (C. H. Young); 2 ♂, 1 ♀, Aweme, Manitoba, September 7, October 4, April 3 (N. Criddle); 8 ♂, Edmonton, Alta., April 23–27, October 1 (K. Bowman), some of these deposited in Bowman's Collection.

Several somewhat larger specimens from the Kaslo region of British Columbia in the Canadian National Collection also belong here, one of these having been erroneously identified by Kearfott as *boscana* Fabr. a species which apparently does not occur in North America.

The present species rather resembles Kennel's figure of *lacordairana* Dup., but the genitalia are quite distinct, if Filipjev's figure of these organs in the above species be correct.

Food Plant. Cornus.

Peronea fuscana B. & B.

Page 328, fig. 7; Page 332, fig. 8.

Peronea fuscana Barnes and Busck, Cont. N. Hist. Lep. N. Am. IV (3), 216. 1920.

The determination of the species is based on information kindly supplied by Mr. A. Busck regarding genitalic characters. It is rather difficult to separate from allied forms such as *cornana* McD. and *heindelana* Fern., but in general the forewings are more contrastingly marked than in either of these species. The ground color is at times quite pale gray and the blackish areas

at base of wing and along costa stand out in consequence quite prominently; there is frequently a strong blackish basal streak or patch present which is characteristic of the species and apparently does not occur in any of the allies.

Fuscana is very closely allied to the European *ferrugana* genitalically, the males showing the same spining of the vesica in both species; in *fuscana*, however, the socii appear considerably broader and the apical hair-tuft of the sacculus is stronger; in the female there are also minor differences which point to specific distinctness.

Genitalia, Male (Six slides examined). Tegumen hood-like; anal sheath with strong ventro-apical projection; socii upright, rather broad and blunt apically; sacculus feebly sinuate with a small projecting chitinous flap at half and a strong, broad, apical hair-tuft; claspers rather broad, aedeagus long, thin, bent; vesica armed with two small, single cornuti, separated by a small, shortly spined piece of chitin (rose-thorn).

Genitalia, Female (One slide examined). Ostium plate with long, nearly straight, lateral lobes, tapering apically; ostium narrow, semicircular; ductus bursae moderately broad and membranous for a short distance proximally, then faintly chitinized to a point near the inception of the ductus seminalis, this area showing faint spiculation distally; narrowing to a fine tube in distal half; signum large, heavily spined.

Distribution. Manitoba, Aweme; Alberta, Edmonton. Specimens have also been examined from Hampton, New Hampshire.

Food Plant. Unknown.

Peronea ferrugana D. & S.

P. Tortrix ferrugana Denis and Schiffermuller, Syst. Verz. 128. 1776.
Acalla ferrugana Kennel, Palaearct. Tort. 93, Pl. V, figs. 40-50. 1909.
Peronea ferrugana Pierce and Metcalfe, Gen. Brit. Tort. 21, Pl. VIII. 1922.
Peronea ferrugana Filipjev, Ann. Mus. Zool. Acad. Sci. U.R.S.S. 522, Pl. XXV, fig. 3, Pl. XXVIII, fig. 3. 1930.
Acleris ferrugana Sheldon, Entomol. LXIV, 60. 1931.

This European species has long been recorded from North America, mainly on the erroneous supposition that *semiannula* Rob. was conspecific with it, which it is not. However, two specimens have been examined in the Canadian National Collection, a male from the Ottawa district and a female from Salt River, North West Territories, the genitalia of which seem to agree with those of European specimens and with the figures of Pierce and Filipjev. These two specimens are very similar to each other in the color and maculation of the forewings, but represent by no means the typical form of *ferrugana*, the ground-color being light gray, slightly speckled with brown specks and with a prominent, more or less tripunctate, brown, costal triangle; they approach closest to Kennel's Fig. 43. More material will be necessary before the specific record can be definitely established, but there seems no real reason why *ferrugana* should not be found in Canada, the food plant, birch, being of such general distribution.

The genitalia, as already stated, are very close to those of *fuscana* B. & B. and are very adequately figured by Pierce; the chief differences in the male genitalia have been commented on under *fuscana;* in the female organs, the lateral lobes of the ostium plate are shorter, thinner and more widely separated; the ostium is narrower; the chitinized portion of the ductus bursae shows a peculiar, crenulate appearance proximally, and is without indication of spicules distally.

Peronea semiannula Rob.

Page 328, fig. 8; Page 332, fig. 9.

Teras semiannula Robinson, Trans. Am. Ent. Soc. II, 282, Pl. VII, fig. 70. 1869.
Peronea stadiana Barnes and Busck, Cont. N. Hist. Lep. N. Am. IV (3) 217. 1920.

The species has been very generally confused and associated with the birch-feeder. A critical study of the original description and figure led to the belief that this association was incorrect and further confirmation was recently secured by Mr. W. J. Brown who, on a visit to the Academy of Natural Sciences, Philadelphia, compared specimens with what appears to be the original type and made careful notes on same. It would seem that the name should be applied to the maple-feeder, *stadiana* B. & B. unfortunately falling as a synonym; of *stadiana* there are not only Paratypes, but also a long series of topotypical specimens in the Canadian National Collection.

The usual color of the forewing in *semiannula* is a light red-brown, but this may pale to fawn color and even occasionally to dull smoky gray; the costal triangle is frequently composed of red-brown scales, is rather indistinct and generally tripunctate in character; it may, however, be dark brown, distinct and either enclosing a pale costal spot or broken up into three, dark, separated spots. Occasional specimens occur with a distinct strigate appearance, due to the presence of small, black streaks in the basal and terminal areas of wing.

Genitalia, Male (Six slides examined). Tegumen hood-like; anal sheath with moderately strong ventro-apical projection; socii upright, somewhat shorter and narrower than in *fuscana* and much as in *ferrugana;* claspers rather short and broad; ventral margin of sacculus slightly angled at base, then feebly sinuate; no chitinous flap, and smaller apical tuft than in *fuscana;* aedeagus narrow, bent, vesica armed with a pair of short apical cornuti and a second longer pair, situated more centrally and with a small chitinous plate between them.

Genitalia, Female (Four slides examined). Lateral lobes of ostium plate moderately long, wide apart, slightly divergent; ostium small, semicircular; ductus bursae almost entirely membranous, with the exception of a short, lightly chitinized ring near proximal end, beyond which is a long sack-like enlargement; terminal portion narrow with inception of ductus seminalis close to bursa; signum large, stellate.

Distribution. Quebec, Meach Lake; Ontario, Ottawa; Alberta, Edmonton; British Columbia, Salmon Arm. Also occurs at Hazelton, Pennsylvania.

Food Plant. Maple (probably).

Peronea gallicolana Clem.

Page 328, fig. 9; Page 332, fig. 10.

Peronea gallicolana Clemens, Proc. Ent. Soc. Phil. III, 516. 1864; Forbes, Mem. 68, Cornell Univ. Agr. Exp. Sta. 487. 1924.
Acleris heindelana Fernald, Am. Nat. XXXIX, 870. 1905.

The typical form of this species, to judge by the original description, is one in which the forewings are rather pale grayish with a distinct and sharply defined, black-brown, costal triangle; the writer has examined a single specimen from Trenton, Ont., which matches this description. The usual form in Canada is the one described later as *heindelana;* the forewings are largely suffused with smoky brown, at times with a slight ruddy tinge; the dark costal triangle is in consequence not prominent, generally better defined in the females than in the males.

Genitalia, Male (Five slides examined). Tegumen hood-like; anal sheath projecting strongly beyond apex of tegumen and with a strong ventro-apical projection; socii upright, rather broader and longer than in allied species; claspers distinctly longer and narrower than in *fuscana;* sacculus feebly sinuate with small chitinous flap at half and weak apical hair-tuft; aedeagus thin, vesica armed with a group of two or three small apical cornuti and a small piece of chitin as well as two further cornuti, slightly larger than the apical ones and more centrally located.

Genitalia, Female (Four slides examined). Ostium plate rather broad with short, parallel, lateral lobes; ostium small, semicircular, situated higher on the plate than in allied species; ductus bursae almost entirely membranous, narrow proximally for a short distance, then expanding into a long oval sack, which shows a slight chitinous thickening and a faint crenulation at one side proximally; beyond the sack is the inception of the ductus seminalis, the remainder of the ductus bursae being short and narrow, signum moderately large, stellate.

Distribution. Ontario, Ottawa, Trenton, Ingersoll; Manitoba, Aweme.

Food Plant. Cone-gall on willow.

Peronea cornana McD.

Page 328, fig. 10; Page 332, fig. 11.

Peronea cornana McDunnough, Can. Ent. 207. 1933; *id.* Can. J. Research, IX, 509. 1933.

The general even olive-gray color of the primaries is rather characteristic of the species. The series on which the name was based evidently belonged to the first generation; specimens taken late in the fall are somewhat larger. In the male genitalia the decumbent socii at once separate the species from its allies.

Genitalia, Male (Six slides examined). Tegumen rather broad, hood-like; anal sheath strongly projecting beyond apex of tegumen, ventro-apical projection much reduced; socii *decumbent,* broadly oval; claspers rather long and narrow, sacculus feebly sinuate, apical hair-tuft well developed; aedeagus

moderately thin, vesica armed with two apical cornuti, arranged one behind the other, and a pair (sometimes three) of similar-sized cornuti, situated more centrally.

Genitalia, Female (Four slides examined). Ostium plate rather narrow, lateral lobes very short, at times scarcely projecting beyond margin of plate; ostium small, semicircular; ductus bursae membranous and moderately broad proximally for a short distance, then feebly chitinized, this chitinous portion forming the neck of a long membranous sack-like expansion, narrowing to a thin tube shortly before entrance into bursa; signum moderately large, stellate.

Distribution. Quebec, Aylmer; Ontario, Bobcaygeon; Manitoba, Aweme; Alberta, Edmonton.

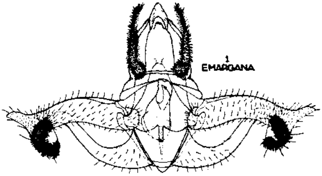
Food Plant. *Cornus.*

Peronea contaminana Hbn.

Tortrix contaminana Hubner, Eur. Schmett. Tort. fig. 142. 1797.
Acalla contaminana Kennel, Palaearc. Tort. 99, Pl. V, figs. 59-61. 1908.
Peronea reticulata Pierce and Metcalfe, Gen. Brit. Tort. 23, Pl. IX. 1922.
Aleimma contaminana Sheldon, Entomol. LXIV, 79. 1931.

In conclusion it might be well to record the occurrence of this European species at Yarmouth, Nova Scotia, where it was found destructive to hawthorn hedges in 1924; it is probably a quite recent introduction.

The subfalcate primaries separate the species at once from other members of the genus and there may be, on this account, some justification for Sheldon's placement in the genus *Aleimma.* The general color of primaries is light to dark brown, more or less reticulate and spotted with darker shades.



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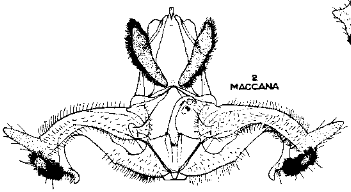

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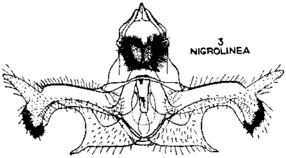

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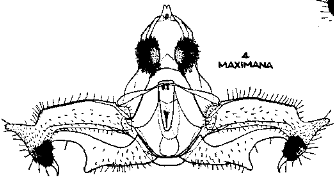

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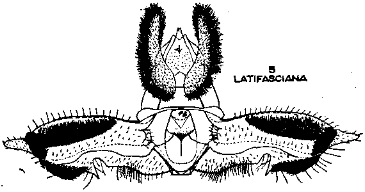

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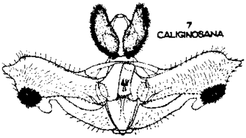
6 PTYCHOGRAMMOS



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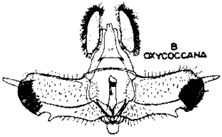

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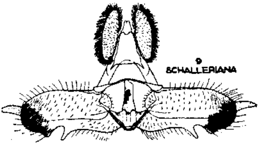

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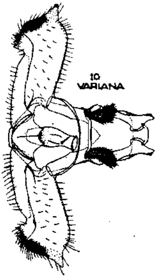

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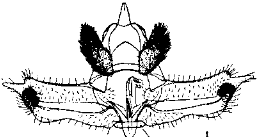
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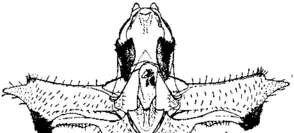
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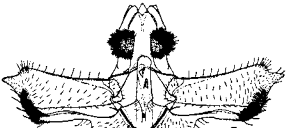
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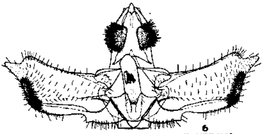
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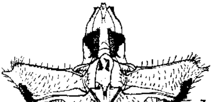
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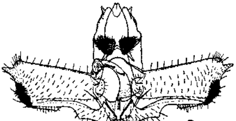
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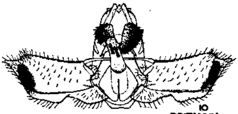
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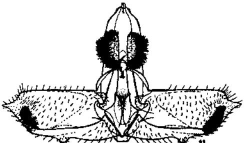
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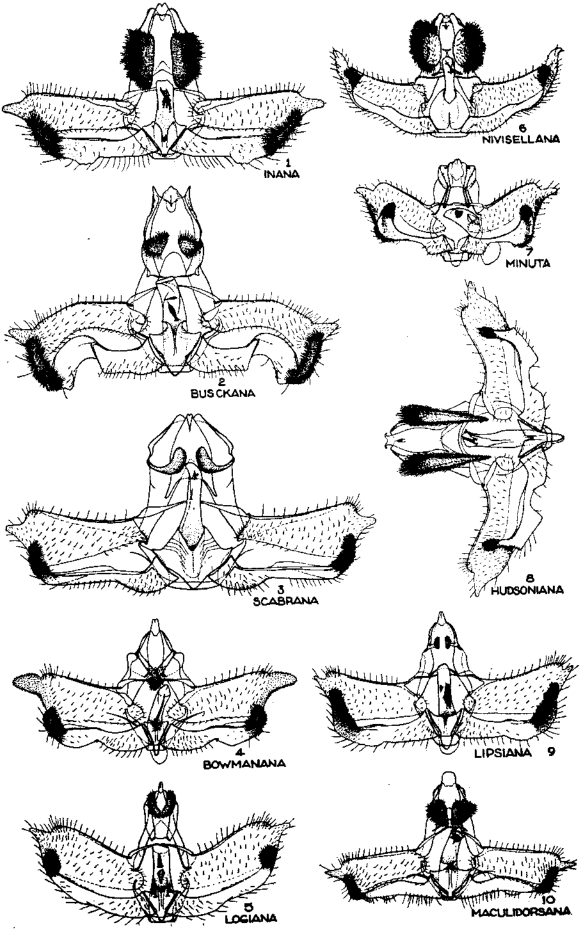
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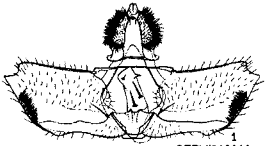

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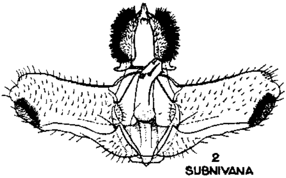

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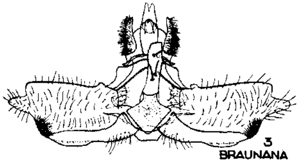

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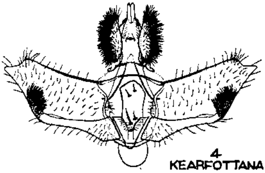

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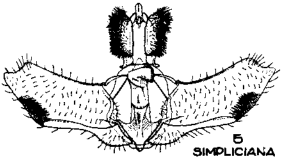

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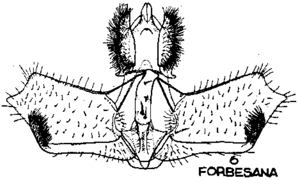

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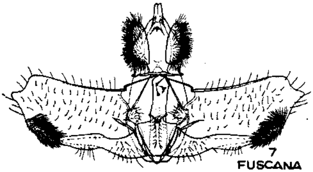

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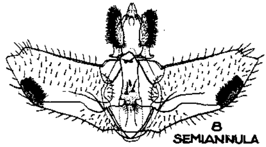

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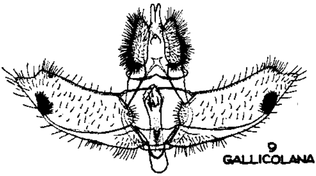

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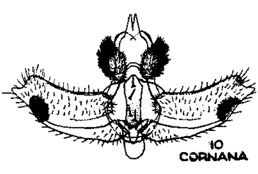

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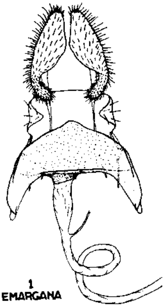

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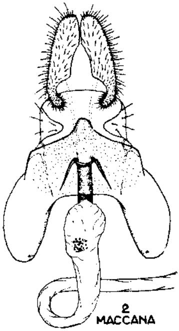

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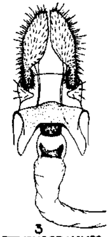

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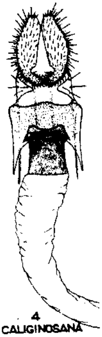

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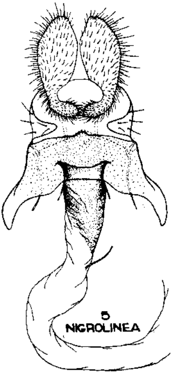

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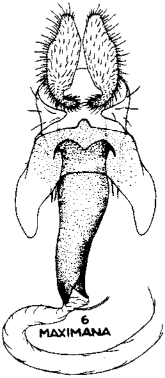

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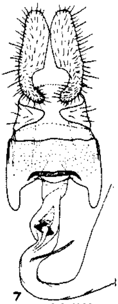

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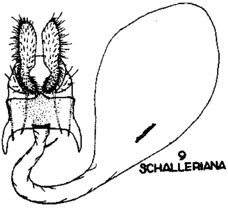

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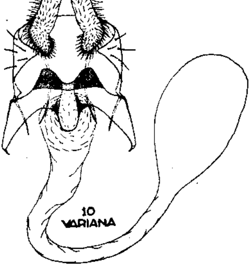

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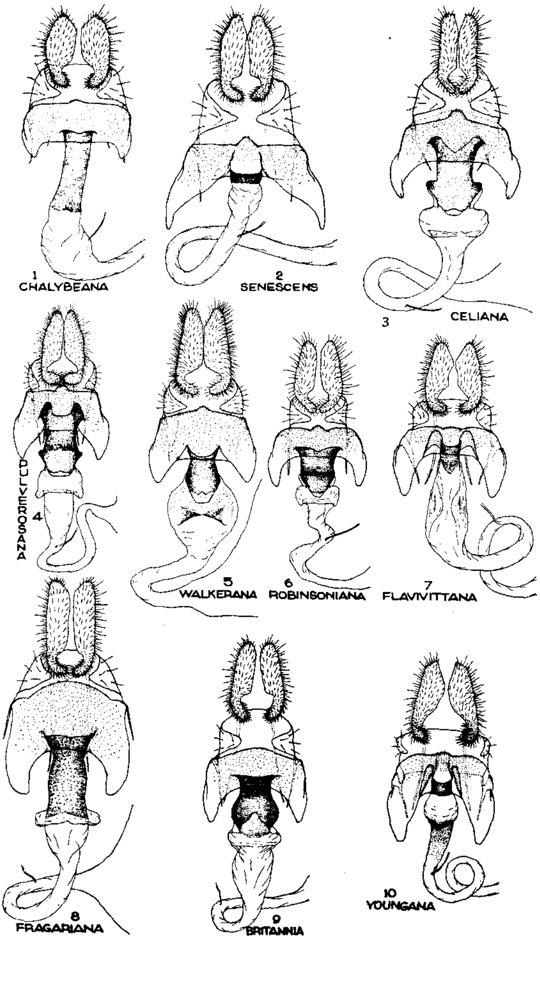
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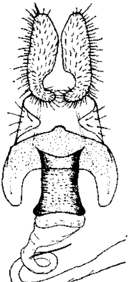

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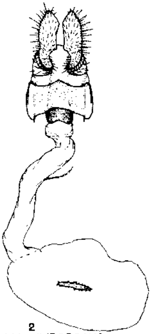

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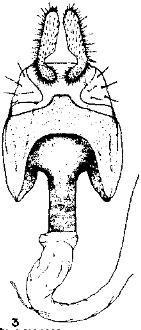

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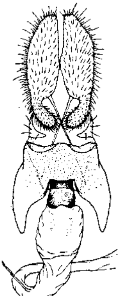

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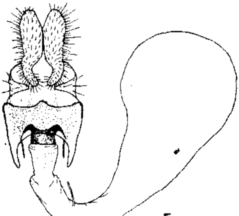

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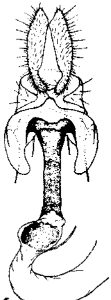

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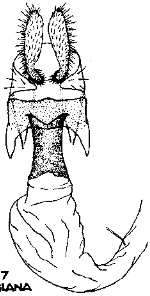

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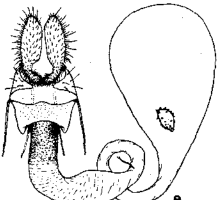

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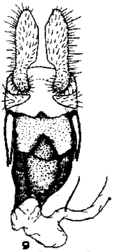

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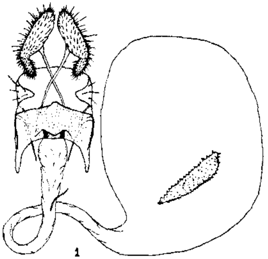

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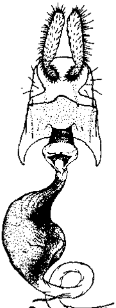

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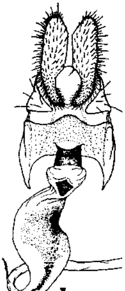

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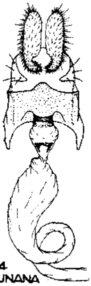

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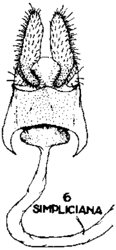

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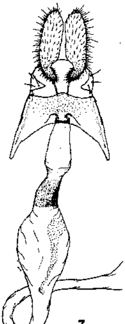

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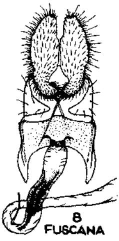

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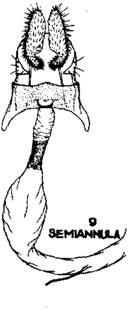

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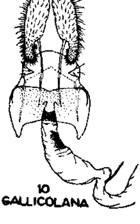

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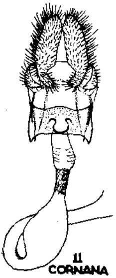

11
CORNANA

F_2 ENDOSPERM DEVELOPMENT IN RELATION TO BREEDING TECHNIQUE WITH INTER-SPECIFIC WHEAT CROSSES[1]

BY J. B. HARRINGTON[2] AND J. B. MARSHALL[3]

Abstract

A comparative study was made of three interspecific wheat crosses with regard to the relationship between seed plumpness and plant character in the F_2 generation. In general, the degree of *vulgare*-ness of the plants was associated with the degree of shrunkenness of the seeds, the most shrunken seeds producing the most *vulgare*-like plants. However, many shrunken seeds did not produce *vulgare*-like plants and many plump seeds did not produce plants resembling the 14-chromosome parental variety. Desirable *vulgare*-like plants were obtainable from each plumpness class, the largest proportion being from the shrunken seeds. Plants from shrunken seeds that were germinated under ideal conditions proved to be no more *vulgare*-like than those which survived sowing under rigorous conditions in the field. The *durum* × *vulgare* cross was high in percentage of shrunken seeds and relatively high in the proportion of *vulgare*-like plants from plump seeds. The *dicoccum* × *vulgare* cross was low in proportion of shrunken seeds and had relatively few *vulgare*-like plants from plump seeds. In these crosses it would appear unnecessary for the breeder to pay attention to differences in F_2 seed plumpness. The *persicum* × *vulgare* cross showed a fair proportion of shrunken seeds but had relatively few *vulgare*-like plants from plump seeds. In this cross it would appear advisable to have an abundance of F_2 seeds and sow only the more shrunken half of them.

Introduction

Hybridization as a plant breeding method is necessarily somewhat complicated and practical methods which tend to simplify and reduce the work in connection with handling large hybrid populations deserve every consideration. Wide species crosses are now used extensively for obtaining various character combinations which are not obtainable from narrow crosses. The necessity for having very large hybrid populations where wide species crosses are concerned has been shown by various investigations.

The breeder working with a large population of a wide cross in wheat has available several methods of procedure and it is not always easy to decide which course to pursue. After parental varieties have been selected, he is faced with the choice of which to use as the female parent. He also has to decide how many F_1 seeds he needs, how to sow the F_1, how to handle the F_2 seeds, and how to carry on from the growing F_2 to his final objective. Experience has shown that widely different results may be obtained with different breeding procedures. As the cost factor is important it is necessary to use an efficient technique, otherwise the size of population required to achieve a satisfactory outcome might not be possible with the available funds.

It is obvious that effective breeding work rests upon an adequate foundation of facts concerning technique. There is much discussion as to the relative

[1] *Manuscript received June 19, 1934.*
Contribution from the laboratories of the University of Saskatchewan, Canada, with financial assistance from the National Research Council of Canada. This study forms a part of a co-operative attack on the problem of cereal rust in Canada, carried on jointly by the National Research Council, the Federal Department of Agriculture and the Universities of Manitoba, Saskatchewan and Alberta.
[2] *Professor of Field Husbandry, University of Saskatchewan.*
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merits of different breeding procedures. The questions that arise very often concern the relative values of the shrunken and plump F_2 seeds. The present study was made with the object of contributing to the technique of handling wide wheat crosses by furnishing answers to some of these questions.

This study is a continuation and an enlargement of a previous study by Harrington (3) in which the relationship between endosperm development and morphologic characters in the F_2 generation of the cross Vernal emmer × Marquis was investigated. The previous study showed the essential differences between plants and populations obtained from shrunken F_2 seeds and those grown from plump F_2 seeds. The results were striking and the question arose: may similar results be expected in other wide crosses? To answer this question a study was made of two quite different crosses, one being *durum* × *vulgare* and the other *persicum* × *vulgare*.

Materials

The material used was obtained from crosses made in connection with actual breeding projects. These crosses involved four species in the following crosses: Iumillo × Marquis, Black Persian × Marquis and Vernal × Marquis. The *durum*, Iumillo Sask. III-25-56, is a strain of high purity selected at the University of Saskatchewan and tracing back to stock originally received from the United States Department of Agriculture. The Marquis crossed with Iumillo is Sask. No. 7, a strain descended directly from the original introduction of Marquis from the Dominion Experimental Farm System of Canada and originated at the University of Saskatchewan by mass selection. The *persicum*, Black Persian Sask. No. 1801, is a purified strain selected at the University of Saskatchewan from the original stock obtained from the United States Department of Agriculture. The Marquis crossed with Black Persian is Sask. No. 1221, a uniform strain from a pedigree selection made at the University of Saskatchewan. The *dicoccum* × *vulgare* cross was described by Harrington (2).

Procedure

A procedure similar to that used by Harrington (2) in 1929 and 1930 on the cross Vernal (*T. dicoccum*) × Marquis (*T. vulgare*) was followed in the present study. Results from the previous study are therefore comparable to those of the present study and will be used here to represent the combination *dicoccum* × *vulgare*.

A random sample of 702 F_2 seeds from the *durum* × *vulgare* cross (Iumillo × Marquis) was divided into three plumpness classes as follows: Plump (Class A), slightly shrunken (Class B) and shrunken (Class C). The sample consisted of 30.9% of Class A, 34.3% of Class B and 34.8% of Class C. The material was hand sown in the breeding nursery in 5-ft. rows, 1 ft. apart, with Marquis and Iumillo every sixth and seventh row. Stands were taken three times, commencing June 8, at intervals of seven days. At maturity each group and parental checks were harvested separately by pulling the

individual plants. A total of 264 F_2 plants was studied in the laboratory with respect to 13 morphologic characters in which the parent varieties differed markedly. A description of the two varieties for each character is given in Table I.

TABLE I

DESCRIPTION OF THE PARENT VARIETIES FOR THE CHARACTERS STUDIED

Character	Marquis (*vulgare*)	Vernal (*dicoccum*)	Iumillo (*durum*)	Black Persian (*persicum*)
Spike form (width ratio of lateral side to dorsi-ventral side)	0.75–0.83	1.5–1.7	0.77–0.85	
Spike compactness (average length of 10 central internodes)	5.1–5.4 mm.	3.4–3.8 mm.	3.5–4.0 mm.	
Stem hollowness (taken 2 cm. below the collar)	Hollow with thin walls	Solid	Solid	Solid
Stem thickness (taken 2 cm. below collar)	1.9–2.1 mm.	0.9–1.0 mm.	1.3–1.6 mm.	1.4–1.7 mm.
Lower width of rachis segment	1.9–2.1 mm.	0.9–1.0 mm.	1.5–1.7 mm.	1.0–1.2 mm.
Rachis hairiness (marginal)	Hairs present, full length	No hairs	Hairs bristle-like at upper eud of inte node	Hairs bristle-like at upper end of internode
Rachis articulation	Difficult	Easy	Difficult	Difficult
Shoulder width	Wide	Narrow	Narrow	Narrow
Glume length-width ratio	*Length almost twice width*	*Length three times width*	*Length three tin es width*	*Length three times width*
Keel sharpness (angle shown by cross section of lower half)	Obtuse	Acute	Acute to 90°	
Beak character	*Short, blunt*		*Prominent and curved*	
Keel prominence	Slight prominence from beak to base of glume		Very prominent	
Brush character	*Numerous hairs*		Few short hairs	
Seed character	Short, plump, with round cheeks and wide deep crease	Long, narrow with angular cheeks and narrow shallow crease	Medium long, with angular cheeks; narrow shallow crease and pointed germ	
Glume adherence	Easily detached	Detached with difficulty		
Awning	Tip awned.	Fully awned		

A similar procedure was followed in the study of the *persicum* × *vulgare* cross. A random lot of 402 F_2 seeds was divided according to the three plumpness classes. There was 17.6% of Class *A* seed, 35.6% of Class *B* and 46.8% of Class *C*. Stands were taken June 8, 16 and 24. *Persicum* and *vulgare* differ by fewer distinctive morphologic characters than do *durum* and *vulgare*. Seven characters were used in this study. The parent varieties are described with respect to these characters in Table I. Other characters could have been used, but the differences were not sufficiently uniform and definite to afford a satisfactory analysis.

The hybrid plants of each cross were classified for each of the contrasted characters listed in Table I into five groups, *viz:* *D*, those resembling the 14-chromosome parent; *V*, those resembling the *vulgare* parent; *I*, those intermediate between *D* and *V*; *ID*, those intermediate between *I* and *D*; and *IV*, those intermediate between *I* and *V*. Most of the data were obtained through visual comparisons of the hybrid with plants of the parental varieties grown close by. Uniform class intervals were maintained for each character as far as it was possible to do so. Occasionally checks on the accuracy of the work were made by actual measurements. In the case of a few characters one or both of the end classes exceeded the interval of the other classes. This was due to the appearance of some forms beyond the parental range and the placing of these segregates in the parental classes rather than in additional classes.

The data on plant character were tabulated for each cross according to the plumpness classes of the seed. For convenience the plants arising from seed plumpness Class *A* will be referred to as Class *A*, those from Class *B* seed as Class *B* and those from Class *C* as Class *C*.

Results on the Relationship between Seed Plumpness and Morphologic Plant Characters in the F_2 of the Crosses *durum* × *vulgare*, *persicum* × *vulgare* and *dicoccum* × *vulgare*

The results on the three crosses should be presented together as they all bear upon the same fundamental problem. Therefore, although the *dicoccum* × *vulgare* data have been published, they will be considered here in relation to the results from the other crosses.

Proportion of each Plumpness Class in the Random Samples

The separation of each F_2 random sample of seeds into three plumpness classes gave the results shown in Table II. The differences between the crosses are quite large. The proportion of shrunken seeds in the *dicoccum* × *vulgare* (this will be referred to as *VM*) F_2 is very much lower and the proportions of plump and slightly shrunken seeds are much higher than in

TABLE II

PROPORTION OF EACH PLUMPNESS CLASS IN THE RANDOM SAMPLES USED

Cross	Cross designation	Distribution of seeds according to plumpness					
		Class *A*		Class *B*		Class *C*	
		No.	%	No.	%	No.	%
durum × *vulgare*	*IM*	217	30.9	241	34.3	244	34.8
persicum × *vulgare*	*MP*	71	17.6	143	35.6	188	46.8
dicoccum × *vulgare*	*VM*	2902	55.5	2144	41.0	183	3.5
Average			34.7		37.0		28.3

the other crosses. The *VM* F_2 was grown in the wet season of 1928, whereas the other F_2 populations were grown in the distinctly dry season of 1930. Plants grown in 1928 had an excellent opportunity to produce kernels with the maximum amount of endosperm development allowed by their chromosome condition. In 1930 the reverse was true. A part of the differences under consideration may be due to this dissimilarity in growing conditions. The difference between the *durum* × *vulgare* and *persicum* × *vulgare* distributions may be due in part to the difficulty of classifying the seeds of widely unlike crosses in the same way.

Seedling emergence

The seedling emergence for the three crosses is shown in Table III. Emergence from Class *A* seeds approached that of the parent varieties in the *VM* F_2 and in the *persicum* × *vulgare* F_2 (this will be called the *MP* cross) but was low, 52.5%, in the *durum* × *vulgare* F_2 (this will be referred to as the *IM* cross). Emergence for Class *B* averaged somewhat lower than for Class *A*. Class *C* emergence was very low in all cases, being approximately half that of Class *A*. The Class *A* emergence in the *IM* cross is strikingly lower than in the *MP* cross which was grown during the same season.

TABLE III

PERCENTAGE SEEDLING EMERGENCE OF THE THREE INTERSPECIFIC CROSSES

Year of study	Cross	Parental variety		F_2 population		
		Vulgare	14-chrom. species	Class *A*	Class *B*	Class *C*
1931	*durum* × *vulgare* (*IM*)	91.6	88.4	52.5	53.9	25.4
1931	*persicum* × *vulgare* (*MP*)	98.0	80.0	80.3	62.2	28.7
1929-30	*dicoccum* × *vulgare* (*VM*)	71.5	67.5	64.0	58.0	35.5
	Average	87.0	78.6	65.6	58.4	29.9

Comparison of Classes A, B and C for Plant Characters

The distributions of the F_2 plants of the *IM* cross with respect to 13 morphologic characters are given in Table IV for each of the seed plumpness classes. The distributions for the three plumpness classes are dissimilar. Considering the average distribution for all characters, Class *A* has nearly twice as large numbers in the *D* and *ID* categories as in *V* and *IV*. Class *C* has about equal numbers in *D* and *ID* as in *V* and *IV*. Class *B* is intermediate with a closer approach to Class *A* than to Class *C*.

The results on the *MP* cross are given in Table V. Here the same tendency is shown except that it is more pronounced, especially in the Class *A* and Class *B* distributions.

TABLE IV

DISTRIBUTION OF THE F_2 PLANTS OF THE *durum* × *vulgare* CROSS WITH RESPECT TO EACH OF 13 MORPHOLOGIC CHARACTERS

Seed plumpness class	Character category	Spike form	Spike compactness	Stem hollowness	Stem thickness	Rachis width	Rachis hairiness	Shoulder width	Glume l-w ratio	Keel sharpness	Beak character	Keel prominence	Brush character	Seed character	Average for all characters
A	*D*	39	64	41	38	24	54	50	48	42	25	29	12	15	37
	ID	16	4	19	14	18	8	16	13	16	18	19	17	42	17
	I	31	8	21	17	20	9	5	12	13	20	28	23	7	16
	IV	8	3	13	11	15	5	10	11	15	13	16	10	19	11
	V	8	23	8	22	25	26	21	18	16	26	10	28	7	18
B	*D*	31	56	34	46	26	58	41	41	31	30	28	15	19	35
	ID	12	7	27	14	20	3	25	14	17	12	25	25	31	18
	I	27	11	34	16	24	5	8	14	21	19	27	10	16	18
	IV	20	12	6	11	16	7	16	15	23	16	22	15	26	16
	V	17	21	6	20	21	34	17	23	15	30	5	30	3	19
C	*D*	10	18	11	15	5	21	10	15	15	17	10	4	5	12
	ID	6	6	11	7	13	2	18	12	9	2	13	10	14	9
	I	9	6	20	4	14	3	9	4	11	12	14	9	8	9
	IV	15	8	8	13	11	9	13	12	11	9	10	10	12	11
	V	15	17	5	16	12	20	5	12	9	15	8	13	6	12

TABLE V

DISTRIBUTION OF THE F_2 PLANTS OF THE *persicum* × *vulgare* CROSS WITH RESPECT TO SEVEN MORPHOLOGIC CHARACTERS

Seed plumpness class	Character category	Stem thickness	Stem hollowness	Rachis width	Rachis hairiness	Shoulder width	Glume l-w ratio	Beak character	Average for all characters
A	*D*	26	25	16	34	35	26	1	23
	ID	4	13	19	3	9	7	5	9
	I	11	8	7	3	3	7	7	7
	IV	5	2	5	3	2	5	14	5
	V	4	2	3	7	1	5	23	6
B	*D*	40	25	17	39	37	31	8	27
	ID	6	23	18	6	19	10	5	12
	I	8	10	16	5	4	6	9	8
	IV	5	7	10	3	5	9	11	7
	V	10	4	8	16	4	13	36	13
C	*D*	25	8	2	13	26	20	0	13
	ID	7	18	11	10	16	10	3	11
	I	7	12	24	13	5	1	4	9
	IV	7	8	9	4	3	10	5	7
	V	5	5	5	11	1	10	39	11

Table VI shows the distributions of the *VM* cross. The preponderance of *D* and *ID* over *V* and *IV* in all three plumpness classes is even stronger here than in the *IM* cross.

TABLE VI

DISTRIBUTION OF THE F_2 PLANTS OF THE *dicoccum* × *vulgare* CROSS WITH RESPECT TO 12 MORPHOLOGIC CHARACTERS

Seed plumpness class	Character category	Spike form	Spike compactness	Stem hollowness	Rachis articulation	Rachis width	Stem thickness	Glume adherence	Rachis hairiness	Shoulder width	Glume shape	Keel sharpness	Awning	*Average for all characters*
A	*D*	17	40	21	14	18	11	6	30	27	23	16	15	20
	ID	16	0	12	23	12	15	8	11	13	12	8	7	11
	I	8	1	10	6	9	14	12	3	5	9	9	2	7
	IV	4	1	3	1	5	5	15	1	1	2	5	6	4
	V	1	4	0	2	2	1	5	1	0	0	8	16	3
B	*D*	37	58	14	28	18	26	22	40	51	37	32	25	32
	ID	29	8	27	19	21	20	18	22	22	34	21	8	21
	I	15	10	26	23	25	23	25	11	13	15	18	17	18
	IV	8	5	16	15	19	14	15	8	3	3	10	28	12
	V	1	9	7	5	7	7	10	9	1	1	9	12	7
C	*D*	15	24	6	17	5	13	8	22	17	11	14	14	14
	ID	22	10	5	18	7	8	13	16	18	15	16	6	13
	I	12	15	25	12	13	17	14	9	13	20	18	5	14
	IV	11	7	20	10	23	10	15	5	11	9	6	23	13
	V	1	5	5	4	13	13	11	9	2	6	7	13	7

TABLE VII

RELATIONSHIP BETWEEN SEED PLUMPNESS AND PLANT TYPE FOR 13 CHARACTERS IN THE F_2 OF THE *durum* × *vulgare* CROSS

Character	Percentage of plants classified as *V* or *IV*			$\frac{\text{Class } C}{\text{Class } A}$	Percentage of plants classified as *D* or *ID*			$\frac{\text{Class } C}{\text{Class } A}$
	Class *A*	Class *B*	Class *C*		Class *A*	Class *B*	Class *C*	
Spike form	16	35	55	3.44	54	40	29	0.54
Spike compactness	26	31	45	1.73	67	59	44	0.66
Stem hollowness	21	11	24	1.14	59	57	40	0.68
Stem thickness	32	29	53	1.66	51	56	40	0.78
Rachis width	39	35	42	1.08	41	43	33	0.81
Rachis hairiness	30	38	53	1.77	61	57	42	0.69
Shoulder width	30	31	33	1.10	65	62	51	0.79
Glume l-w ratio	29	36	44	1.52	60	51	49	0.82
Keel sharpness	30	36	36	1.20	57	45	44	0.77
Beak character	38	43	44	1.16	42	39	35	0.83
Keel prominence	25	25	33	1.32	47	49	42	0.89
Brush character	42	47	50	1.19	32	42	30	0.94
Seed character	29	31	40	1.38	63	53	42	0.67
Average	30	33	42	1.40	54	50	40	0.74

NOTE.—*The* χ^2 *goodness of fit test applied to* $\frac{\text{Class } C}{\text{Class } A}$ *for % V plus IV versus* $\frac{\text{Class } C}{\text{Class } A}$ *for % D plus ID shows enormous odds that the two sets of ratios are not similar.*

In general, the more shrunken the F_2 seed the greater is the *vulgare*-ness of the plants for the characters studied.

The results given in Tables IV, V and VI are more easily analyzed when summarized on a percentage basis. Such a summary is clarified by considering that a plant is *vulgare*-like for a given character if it is recorded as *V* or *IV* for that character and conversely that its inclusion in either of the categories *D* or *ID* designates its resemblance to its 14-chromosome grandparent. The data were therefore summarized on this basis and are shown in Tables VII, VIII and IX.

TABLE VIII

Relationship between seed plumpness and plant type for seven characters in the F_2 of the *persicum* × *vulgare* cross

Character	Percentage of plants classified as *V* or *IV*			Class *C* / Class *A*	Percentage of plants classified as *D* or *ID*			Class *C* / Class *A*
	Class *A*	Class *B*	Class *C*		Class *A*	Class *B*	Class *C*	
Stem thickness	18	22	24	1.33	60	67	63	1.05
Stem hollowness	8	16	26	3.25	76	70	51	0.67
Rachis width	16	26	27	1.69	70	51	26	0.37
Rachis hairiness	20	28	29	1.45	84	65	45	0.54
Shoulder width	6	13	8	1.33	88	81	82	0.93
Glume l-w ratio	20	50	39	1.95	66	59	59	0.89
Beak character	74	68	86	1.16	12	19	6	0.50
Average	23	32	34	1.48	65	59	47	0.72

Note.—*See footnote of Table VII.*

TABLE IX

Relationship between seed plumpness and plant type for 12 characters in the F_2 of the *dicoccum* × *vulgare* cross

Character	Percentage of plants classified as *V* or *IV*			Class *C* / Class *A*	Percentage of plants classified as *D* or *ID*			Class *C* / Class *A*
	Class *A*	Class *B*	Class *C*		Class *A*	Class *B*	Class *C*	
Spike form	11	10	20	1.82	72	73	61	0.85
Spike compactness	11	16	20	1.82	88	73	56	0.64
Stem hollowness	7	26	41	5.86	72	46	18	0.25
Rachis articulation	7	22	23	3.29	80	52	57	0.71
Rachis width	15	29	59	3.93	65	43	20	0.31
Stem thickness	13	23	38	2.92	57	51	34	0.60
Glume adherence	43	28	43	1.00	30	44	34	1.13
Rachis hairiness	4	19	23	5.75	89	69	62	0.70
Shoulder width	2	4	21	10.50	88	81	57	0.65
Glume shape	4	4	25	6.25	76	79	43	0.57
Keel sharpness	28	21	21	0.75	52	59	49	0.94
Awning	48	44	59	1.23	48	37	33	0.69
Average	16	21	33	2.06	68	59	44	0.65

Note.—*See footnote of Table VII.*

In the *durum* × *vulgare* cross, Class *C* was more *vulgare*-like than Class *B* in all of the 13 characters and Class *B* was more *vulgare*-like than Class *A* in nine characters. In the *persicum* × *vulgare* cross, Class *C* was more *vulgare*-like than Class *B* in five of the seven characters and Class *B* was more *vulgare*-like than Class *A* in six characters. In the *dicoccum* × *vulgare* cross Class *C* was more *vulgare*-like than Class *B* in 11 of the 12 characters and Class *B* was more *vulgare*-like than Class *A* in 8 of the 12 characters.

The ratio of Class *C* to Class *A* for percentage of *vulgare*-ness (*V* plus *IV*) is shown in the fourth column of Tables VII, VIII and IX. The Class *C* to Class *A* ratio is given in the eighth column for the percentage resemblance to the 14-chromosome parent (*D* plus *ID*). The difference between the two columns of ratios is highly significant in each cross and shows clearly the wide difference in character expression between Classes *A* and *C*

Character of Individual Plants with Respect to Classes A, B and C

The foregoing presentation of the results has been on the basis of population characteristics and tendencies, but it is perhaps even more important to consider the data from the viewpoint of the character of individual plants. This was done in the *durum* × *vulgare* cross by taking *ID* and *D* as indicating *durum*-like character and *IV* and *V* as representing *vulgare*-like character. Category *I*, being a neutral class, was not considered. Plants classified as *D* or *ID* for at least two more characters than as *IV* or *V*, were considered to be *durum*-like. Similarly plants described as *IV* or *V* for at least two more characters than as *D* or *ID*, were called *vulgare*-like. The ratio of *durum*-like to *vulgare*-like was then averaged with the ratio obtained by considering all plants with a majority of *D* and *ID* over *IV* and *V* as *durum*-like, and all those with a majority of *IV* and *V* over *D* and *ID* as *vulgare*-like. The same method was used in the *persicum* × *vulgare* and *dicoccum* × *vulgare* crosses. The relative proportions of plants classified as either *vulgare*-like or like the 28-chromosome parent variety in each plumpness class for each cross are summarized in Table X.

TABLE X

PLANT TYPE RATIOS IN THE THREE INTERSPECIFIC CROSSES

Plant type ratio	Plumpness classes for F_2 seed *A*	*B*	*C*
persicum : *vulgare*	9.3 : 1.0	3.1 : 1.0	2.2 : 1.0
durum : *vulgare*	2.6 : 1.0	1.8 : 1.0	.88 : 1.0
dicoccum : *vulgare*	12.1 : 1.0	5.3 : 1.0	2.2 : 1.0

The results show that for all three crosses there is a pronounced tendency toward more *vulgare*-ness in Class *B* than in Class *A*, and in Class *C* than in Class *B*. In the *VM* cross, Class *A* is nearly six times as *dicoccum*-like as Class *C* but in the *MP* and *IM* crosses, Class *A* is only four and three times as *persicum*-like and *durum*-like, respectively, as Class *C*. Comparing the results for Class *B* and Class *C*, the slightly shrunken seeds produced on the average about half as many *vulgare*-like plants as did the shrunken seeds. As with field emergence, the results furnish evidence that crosses of *vulgare*

TABLE XI

PROPORTION OF *vulgare*-LIKE PLANTS IN EACH PLUMPNESS CLASS

Cross	Plumpness classes			
	A, %	*B*, %	*C*, %	Average, %
IM	27.8	35.7	53.2	38.9
MP	9.7	24.4	31.3	21.8
VM	7.6	15.9	31.3	18.3

with different 28-chromosome wheats do not react alike although certain general tendencies are found in each of them.

The differences as to plant type in the three crosses and the three plumpness classes are seen clearly when the ratios of Table X are given as percentages of *vulgare*-like plants. This is done in Table XI.

Vulgare-ness of the Vulgare-like Plants in each Plumpness Class

The *vulgare*-like plants are not equally *vulgare-like* since some are *vulgare*-like in all characters, whereas others may be *vulgare*-like in a bare majority of characters, therefore, it is of interest to examine the character of these plants to determine the relationship between the degree of *vulgare*-ness and the plumpness of the F_2 seeds. In Table XII this relationship is revealed for the three crosses. The data on the *vulgare*-like plants are summarized according to the three plumpness classes, and in each case the ratio of *D* plus *ID* to *IV* plus *V* is determined. In the *IM* cross, *vulgare*-like plants of Class *C* were less *vulgare*-like than the *vulgare*-like plants of Classes *B* or *A*. In the *VM* cross the opposite was true. In the *MP* cross the greatest *vulgare*-ness was in the Class *B* *vulgare*-like plants; however the least *vulgare*-ness was in the *vulgare*-like plants of Class *A* as in the *VM* cross. The *IM* results are much at variance with those of the other two crosses and serve to accentuate the differences already noted in the behavior of the three crosses. It is noticeable that the *MP* and *VM* crosses both had very few *vulgare*-like plants in Class *A* and these plants were less *vulgare*-like as groups than any of the other groups shown in Table XII.

TABLE XII

THE RELATIONSHIP BETWEEN F_2 SEED PLUMPNESS AND THE DEGREE OF *vulgare*-LIKE CHARACTER IN THE *vulgare*-LIKE F_2 PLANTS OF THREE INTERSPECIFIC CROSSES

Cross	Plumpness class	No. of *vulgare*-like plants	Character categories					Ratio of "14-chromosome-like" to *vulgare*-like character
			D	*ID*	*I*	*IV*	*V*	
IM	*A*	28	23	23	67	65	180	1.0 : 5.3
	B	38	35	42	118	121	174	1.0 : 5.1
	C	28	26	43	75	93	121	1.0 : 3.1
MP	*A*	5	2	6	5	7	15	1.0 : 1.8
	B	18	11	12	17	27	59	1.0 : 3.7
	C	17	15	11	19	37	37	1.0 : 2.8
VM	*A*	4	10	5	7	12	14	1.0 : 1.9
	B	15	23	25	46	59	38	1.0 : 2.0
	C	18	25	20	61	70	56	1.0 : 2.8

The Effect of Taking Special Care of Seedlings from Shrunken Seeds

In the opinion of some breeders, the plants from shrunken seeds in a wide interspecific wheat cross should be given special care. The assumption is that various interspecific character combinations which occur in plants from shrunken seeds, do not occur in plants from the plumper seeds. While the present study has given no evidence that this is true, it was thought worthwhile to see whether or not a population given special seedling care would be more *vulgare*-like than one given ordinary field treatment.

A lot of 150 very shrunken F_2 seeds of the cross Vernal emmer × Marquis was divided into two parts, one part being sown shallow in the greenhouse in wooden flats, and the other portion being sown directly in the nursery without any special care. The greenhouse conditions were fairly ideal for the development of wheat seedlings, whereas the nursery conditions were rather severe, the spring being dry and windy, and the nights cold. When the greenhouse plants were well started, they were carefully transplanted to the nursery. The results of the experiment are given in Table XIII.

TABLE XIII

COMPARISON OF PLANTS STARTED IN GREENHOUSE AND IN NURSERY

Plants started in	No. seeds sown	Stand in June	Emergence, %	Ratio of *dicoccum*-like to *vulgare*-like plants
Greenhouse	75	33	40.0	4.6 : 1.0
Nursery	75	25	33.3	4.8 : 1.0

The results show clearly that the plants from shrunken seeds started in the greenhouse gave approximately the same proportion of *vulgare*-like plants as those sown directly in the field. The stand was better for the greenhouse plants, but this was far outbalanced by the large amount of labor involved in the greenhouse care, and the transplanting. The greenhouse group of plants gave a total of three *vulgare*-like plants. An equal number of *vulgare*-like plants occurred in the nursery-sown group. These two sets of *vulgare*-like plants were about equally *vulgare*-like. The experiment indicated that special care of the shrunken seeds was a waste of time.

Discussion

It has been proved by Harrington and Smith (3), Harrington (1), McFadden (4), Stevenson (5) and others, that extremely large hybrid populations are necessary if reasonable hope for success in wide wheat crosses is to be entertained. It is of considerable importance, therefore, that suitable methods of dealing with such crosses be developed. The wide variation in the endosperm development of the F_2 seeds immediately arrests attention. If the use of any definite portion of these seeds, as segregated on the basis of the degree of "plumpness," is better than the use of all the seeds or of some other part, it

is important to know it. Harrington (2) showed that in a *dicoccum* × *vulgare* cross the shrunken seeds produced a larger proportion of *vulgare*-like plants than did the plumper seeds. The present study has demonstrated the same tendency in two other interspecific crosses.

The establishment of the fact that there is a definite general relationship between the degree of endosperm development of the seed and the morphologic type of the resulting plant raised the question of the closeness of this relationship and its practical implications. The relationship was not found to be close; many plump seeds produced *vulgare*-like plants and many shrunken seeds produced plants resembling the 14-chromosome parent species. While the *vulgare*-like plants from shrunken seeds were distinctly more *vulgare*-like than those from plump seeds, the latter were found to be as *vulgare*-like as many of those from shrunken seeds. Furthermore, the shrunken seeds that were coaxed to grow under ideal conditions produced plants that were no more *vulgare*-like than those from shrunken seeds sown directly in the field nursery.

A study of the plants from each seed plumpness class of each cross revealed a wide assortment of character combinations. There was every indication that *vulgare* plants with desired character resemblances to the 14-chromosome parent would be obtainable from any of the plumpness classes, providing the population was very large.

The general conclusions from the breeding standpoint are that no attention need be paid to the endosperm development of the F_2 seeds, for while plants from the shrunken seeds are more *vulgare*-like than those from the other seeds, the shrunken seeds make up less than a third of the whole population and do not yield character combinations unobtainable from plants from plump seeds. However, examination of the results from the different crosses indicates that each cross must be considered by itself. The *durum* × *vulgare* cross *(IM)* was relatively high in proportion of shrunken seeds, relatively low in germination of plump seeds and relatively high in the total proportion of *vulgare*-like plants as well as in the proportion of *vulgare*-like plants from plump seeds. In this cross, there would appear to be no good reason for paying attention to the plumpness of the F_2 seeds.

The *persicum* × *vulgare* cross *(MP)* was (like *IM*) distinctly high in proportion of shrunken seeds, high also (unlike *IM*) in the germination of the plump seeds, low compared to *IM* in the total proportion of *vulgare*-like plants and very low in the proportion of *vulgare*-like plants from plump seeds. In this cross, the high proportion of shrunken and slightly shrunken seeds and the large proportion of *vulgare*-like plants from these two seed classes seem to favor the paying of special attention to the shrunken F_2 seeds. The high germination of the plump seeds is an offsetting factor. On the whole, in a cross with this behavior, it might be advisable to classify the F_2 seeds into two groups, the plump and shrunken, and sow only the latter group.

The *dicoccum* × *vulgare* cross *(VM)* studied by Harrington (2) was very low in proportion of shrunken seeds and higher than either of the other

crosses in percentage germination, using the germination of the 14-chromosome check varieties as a standard, but in proportion of *vulgare*-like plants this cross was even lower than *MP*, particularly in Class *B*. The results suggest that in a cross of this kind the large proportion of plump seeds is offset by its low degree of *vulgare*-ness and the low proportion of shrunken seeds is offset by its fairly high degree of *vulgare*-ness. On the whole then, there seems to be little reason for paying attention to F_2 seed plumpness in this cross.

No doubt the use of other species would reveal additional differences among interspecific crosses. Various investigators have shown that wide genetic differences exist between different wheats of the same chromosome number. A comprehensive understanding of the behavior of crosses between 14- and 21-chromosome wheats would necessitate a vast amount of work involving at least a dozen representatives of each chromosome group. Therefore, in a limited study, it is necessary to restrict the conclusions specifically to the material used. However, the present work is sufficiently wide in its scope to suggest a general procedure that is not likely to be far wrong, *viz.*, the use of a *large* population of F_2 seeds sown with ordinary care in a field nursery.

Summary

1. A study was made of the relationship between endosperm development and plant type in the F_2 generation of three interspecific wheat crosses: Iumillo *(durum)* × Marquis *(vulgare)*, Marquis × Black Persian *(persicum)* and Vernal *(dicoccum)* × Marquis, the latter from data published in 1931 (2).

2. The F_2 seeds of each cross were divided for convenience into three groups on the basis of seed plumpness, *viz.*, plump (Class *A*), slightly shrunken (Class *B*) and shrunken (Class *C*). The proportions of seeds in the three classes when averaged for the three crosses were 35, 37 and 28%.

3. Seed plumpness proved to be closely related to seedling emergence.

4. In each of the crosses, plants from plump seeds were less *vulgare*-like than those from slightly shrunken seeds and the latter were less *vulgare*-like than plants from shrunken seeds.

5. Each of the crosses showed distinctive behavior as follows: the Iumillo × Marquis cross *(IM)* resembled the Marquis × Black Persian cross *(MP)* in the distribution of seeds according to the three plumpness classes. In seedling emergence the *IM* cross was very low in Class *A*, whereas the *MP* cross was high and the Vernal × Marquis cross *(VM)* was fairly high. In the F_2 distribution for each of the morphologic characters, the *IM* cross differed distinctly from the other two by showing a greater proportion of *vulgare*-ness in each class. Consideration of the type of each F_2 plant pointed to a greater similarity between the *MP* and *VM* crosses than between either of these and the *IM* cross. The *MP* cross differed from *VM* in that the latter showed much less *vulgare*-ness in Class *A*.

6. From the standpoint of breeding for *vulgare*-like hybrids possessing only one or two characters of the 14-chromosome species, the results indicated that the plumper half of the F_2 seeds of the *persicum* × *vulgare* cross could well be discarded in favor of using the more shrunken half, but in the case of the *durum* × *vulgare* and *dicoccum* × *vulgare* crosses, the best procedure would be to sow the F_2 seeds without regard to their plumpness.

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CHIASMA FREQUENCY IN SPECIES AND SPECIES HYBRIDS OF *AVENA*[1]

By Jane D. Spier[2]

Abstract

(i) Chiasma frequency at metaphase has been studied in the following species and species hybrids: *A. strigosa* Schreb.; *A. brevis* Roth; *A. Wiestii* Steud.; *A. barbata* Pott; *A. abyssinica* Hochst. (2 strains); *A. sterilis* L. (2 strains); *A. sativa* L. var. Radnorshire Sprig; a triploid hybrid, *A. barbata* × *A. strigosa;* a pentaploid hybrid, *A. abyssinica* "naine" × *A. sterilis* maxima; and a hexaploid hybrid, *A. sterilis* (white) × *A. sativa* var. Radnorshire Sprig.

(ii) The various factors that may affect chiasma frequency are discussed and it is concluded that there may be some parallelism between closeness of relationship and similarity in chiasma formation, as genetical control is one factor affecting the process. The results are examined in the light of this conclusion.

(iii) The homogeneity of species in respect to proportion of bivalents with zero, one, two, three, or four chiasmata is tested by means of χ^2. The test indicates no significant difference between the closely related diploid species, *A. brevis* and *A. strigosa*, but both differ significantly from the less closely related *A. Wiestii*. The differences between the tetraploid species are of questionable significance, those between the hexaploids, insignificant. It is suggested that the lack of significant differences among these last-mentioned forms may be due to a multiplication, in the polyploid, of genes favoring high chiasma frequency. In this way a maximum effect might be brought about, and interspecific differences with respect to this character would consequently be lacking.

(iv) High chiasma frequency in the hybrid might be regarded as strong evidence of homology, and consequently of relationship between parental forms. The triploid hybrid, *A. barbata* × *A. strigosa* shows a high chiasma frequency at metaphase, a condition consistent with the close relationship of the parental forms. The pentaploid hybrid, as might be expected, has a low chiasma frequency. The chiasma frequency of the hexaploid hybrid, *A. sterilis* × *A. sativa* var. Radnorshire Sprig does not differ significantly from that of its low-frequency parent, *A. sterilis*. Cytological observations in this hybrid, therefore, reveal only a slight degree of non-homology between parental chromosomes. It is noted that there are certain characters of *A. sativa* var. Radnorshire Sprig that suggest affinity with *A. byzantina*, a species commonly associated with *A. sterilis* in relationship schemes.

The interspecific relationships that exist within the section *Euavena* are clearly indicated by the morphological characteristics of the different species and by serological reactions between them. The genealogical tree of Thellung (17) shows these interspecific relationships in a generally accepted form.

Nishiyama (12) made a cytological study of various interspecific hybrids of *Avena*. He expressed the degree of relationship between parental species by the ratio in the hybrid of:

$$\frac{\text{number of bivalents found}}{\text{number of bivalents expected}}\,.$$

A full affinity between parental species is indicated by a value of one.

According to Nishiyama (12), those species which are closely related in the genealogical tree of Thellung (17) showed cytological relationships approaching unity, while more distantly related species gave, on the whole, cytological relationships that were considerably lower than one.

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It is difficult to determine the degree of homology that exists between two chromosomes. For many organisms, it is best estimated at present by comparative studies of chiasma frequency. As Darlington (3) points out, there are difficulties in the way of such a procedure. Not only homology, but also the arrangement of homologous parts is involved. Moreover, competition in pairing in a polyploid may result in lowered chiasma frequency, and it seems evident that environmental or developmental conditions may affect prophase pairing or chiasma formation (Kihara (10), Hollingshead (7)).

Nishiyama's (12) findings are based upon a rough approximation to chiasma frequency, but his method reveals only gross differences in the species hybrids. It was decided, therefore, to study chiasma frequency at metaphase of various species and interspecific hybrids of *Avena* in order to detect, if possible, any significant differences with respect to this characteristic.

Material and Methods

The species and species hybrids shown in Table I were studied.

TABLE I

SPECIES AND SPECIES HYBRIDS STUDIED

No.	Species	Chrom. No. (2*N*)	Origin	Year grown
1	*A. brevis* Roth	14	Prof. Fraser, Cornell Univ., Ithaca, N.Y.	1932
2	*A. Wiestii* Steud.	14	Dr. T. R. Stanton, U.S.D.A., C.I. No. 1994.	1932
3	*A. strigosa* Schreb.	14	From Spain, via Bureau of App. Bot. and Plant Breeding, U.S.S.R.	1932
4	*A. barbata* Pott	28	Dr. T. R. Stanton, U.S.D.A., C.I. No. 2467.	1932
5	*A. abyssinica* Hochst. ("naine")	28	Ch. Crepin, Clermont, Ferrand. Originally found by Vilmorin.	1932
6	*A. abyssinica* Hochst. (gray)	28	Dr. T. R. Stanton, U.S.D.A., C.I. No. 2109-6.	1930
7	*A. sterilis* L. (maxima)	42	H. Hunter, School of Agriculture, Cambridge.	1932
8	*A. sterilis* L. (white)	42	Axel Pedersen, Agr. H. School, Copenhagen.	1932
9	*A. sativa* L. var. (Radnorshire Sprig)	42	E. T. Jones, Aberystwyth.	1932
10	3*x* hybrid (No. 4 × No. 3)	21		1929
11	5*x* hybrid (No. 5 × No. 7)	35		1929
12	6*x* hybrid (No. 8 × No. 9)	42		1930

According to Emme (4), the question of the chromosome number of the true *A. Wiestii* Steudel is as yet unsettled. Huskins (8) reports that the haploid chromosome number of this species is seven. Emme (4) remarks that as he gives no information on distribution or specific characteristics of this wild form, she is uncertain of its identity. The form investigated by Huskins (8) is the *A. Wiestii* Steudel of this paper. It is described by Stanton and Dorsey (16).

The species hybrids and *A. abyssinica* (gray) were grown at the John Innes Horticultural Institute, and the remaining pure species at Macdonald College, P.Q.

Permanent aceto-carmine smears were made of microspore mother cells of all material except the triploid hybrid. The triploid hybrid stamens were fixed in Kihara's Carnoy-Flemming, embedded in paraffin and sectioned at 14 μ. The sections were stained by Newton's iodine crystal violet method. Metaphase plates of the first maturation division were studied and the number of chiasmata counted in cells whose full chromosome complement could clearly be distinguished.

Observations

Tables II to V show the number of bivalents per cell with zero, one, two, three or four chiasmata in diploid, tetraploid and hexaploid species and in triploid, pentaploid and hexaploid hybrids.

(a) Diploid Species

Chiasma frequencies of *A. brevis* and *A. strigosa* are very similar (Table II). The average number of chiasmata per cell in 20 cells is 15.30 in the former, 15.15 in the latter, and in both species the proportions of bivalents with zero, one, two, or three chiasmata are alike.

TABLE II

CHIASMA FREQUENCY N 2*x* SPECIES OF *Avena*

Cell No.	Chiasmata per bivalent per cell											
	A. strigosa				*A. Wiestii*				*A. brevis*			
	0	1	2	3	0	1	2	3	0	1	2	3
1	—	1	3	3	—	—	5	2	—	—	3	4
2	—	—	4	3	—	—	6	1	—	1	5	1
3	—	2	4	1	—	—	7	—	—	—	5	2
4	—	1	5	1	—	—	6	1	—	1	3	3
5	—	1	4	2	—	1	6	—	—	1	6	—
6	—	1	5	1	—	1	4	2	—	—	6	1
7	—	1	3	3	—	1	6	—	1	1	2	3
8	—	1	5	1	—	2	5	—	—	—	4	3
9	—	2	2	3	—	1	6	—	—	2	5	—
10	—	1	4	2	—	2	5	—	—	—	3	4
11	—	—	5	2	—	2	5	—	—	1	4	2
12	—	2	2	3	—	1	6	—	—	3	2	2
13	—	—	6	1	—	2	5	—	—	—	6	1
14	—	1	4	2	—	2	5	—	—	2	4	1
15	—	—	4	3	—	1	6	—	—	—	6	1
16	—	—	6	1	—	1	5	1	—	1	3	3
17	—	—	5	2	—	1	6	—	—	—	4	3
18	—	1	4	2	—	1	6	—	—	—	5	2
19	1	—	5	1	—	1	6	—	—	—	5	2
20	—	—	4	3	—	2	5	—	—	1	2	4
Total	1	15	84	40	0	22	111	7	1	14	83	42
Average per cell	.05	.75	4.2	2.0	0	1.1	5.55	.35	.05	.70	4.15	2.1
Mean no. chiasmata per cell	15.15 ± 0.24				13.25 ± 0.24				15.30 ± 0.24			

TABLE III

CHIASMA FREQUENCY IN $4x$ SPECIES OF *Avena*

Cell No.	Chiasmata per bivalent per cell											
	A. barbata				*A. abyssinica* ("naine")				*A. abyssinica* (gray)			
	0	1	2	3	0	1	2	3	0	1	2	3
1	—	—	11	3	—	3	10	1	—	2	11	1
2	—	—	12	2	1	1	10	2	—	2	10	2
3	—	2	8	4	—	5	5	4	—	4	9	1
4	—	—	12	2	—	4	8	2	—	1	8	5
5	—	—	12	2	—	4	9	1	—	2	9	3
6	—	4	6	4	—	3	11	—	—	1	12	1
7	—	4	7	3	—	3	7	4	—	4	8	2
8	—	—	—	—	—	4	9	1	—	2	12	—
9	—	—	—	—	—	3	9	2	—	1	11	2
10	—	—	—	—	—	3	11	—	—	2	9	3
11	—	—	—	—	—	3	10	1	—	4	9	1
12	—	—	—	—	1	2	9	2	—	4	9	1
13	—	—	—	—	—	4	8	2	—	—	13	1
Total	—	10	68	20	2	42	116	22	—	29	130	23
Average per cell	—	1.43	9.71	2.86	.15	3.23	8.93	1.69	—	2.23	10.0	1.77
Mean no. chiasmata per cell	29.42 ± 0.40				26.15 ± 0.30				27.54 ± 0.30			

TABLE IV

CHIASMA FREQUENCY IN $6x$ SPECIES OF *Avena*

Cell No.	Chiasmata per bivalent per cell											
	A. sterilis $5n$ parent				*A. sterilis* $6n$ parent				*A. sativa*, var. Radnorshire Sprig			
	0	1	2	3	0	1	2	3	0	1	2	3
1	—	1	13	7	—	1	13	7	—	1	9	11
2	—	2	11	8	—	1	11	9	—	3	10	8
3	—	3	8	10	—	2	12	7	—	—	17	4
4	—	3	12	6	—	3	14	4	—	—	14	7
5	—	1	11	9	—	3	14	4	—	1	13	7
6	—	—	14	7	—	3	11	7	—	—	16	5
7	—	2	14	5	—	1	16	4	—	—	14	7
8	—	2	14	5	—	—	—	—	—	—	—	—
9	—	1	11	9	—	—	—	—	—	—	—	—
Total	—	15	108	66	—	14	91	42	—	5	93	49
Average per cell	—	1.65	12.0	7.33	—	2	13	6	—	.71	13.29	7.00

TABLE V

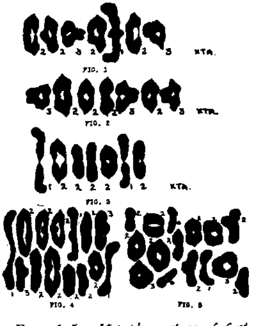
CHIASMA FREQUENCY IN $3x$*, $5x$ AND $6x$ SPECIES HYBRIDS, *Avena*

Cell No.	Chiasmata per bivalent per cell												
	$6x$ hybrid				$5x$ hybrid				$3x$ hybrid				
	0	1	2	3	0	1	2	3	0	1	2	3	4
1	—	2	14	5	8.5	9	—	—	3.0	1	.5	5	1
2	—	1	17	3	9.5	7	1	—	2.5	2	—	6	—
3	—	5	11	5	10.5	7	—	—	2.5	3	1	4	—
4	—	4	10	7	7.5	10	—	—	2.5	1	3	3	1
5	—	2	13	6	7.5	10	—	—	3.5	—	2	5	—
6	—	2	12	7	10.5	6	1	—	3.5	1	2	4	—
7	—	1	16	4	9.5	6	2	—	3.5	1	5	1	—
8	—	1	13	7	8.5	8	1	—	2.5	1	3	4	—
9	—	2	12	7	8.5	8	1	—	3.5	—	3	4	—
10	—	3	11	7	9.5	7	1	—	3.5	2	2	3	—
Total bivalent	—	23	129	58	90.0	78	7	—	30.5	12	21.5	39	2
Average per cell	—	2.3	12.9	5.8	9	7.8	.7	—	3.05	1.2	2.15	3.9	.2
% per cell (hybrids)	—	11	61	28	51.5	44.5	4.0	—	29	11	21	37	2
% per cell (♂ parent)	—	4	63	33	—	8	57	35	—	11	60	28	—
% per cell (♀ parent)	—	9	62	29	1	22	64	13	1	10	70	20	—

* *A. sterilis (white)* × *A. sativa var. Radnorshire Sprig, 6x hybrid; A. abyssinica ("naine")* × *A. sterilis maxima, 5x hybrid; A. barbata* × *A. strigosa, 3x hybrid.*

A. Wiestii shows a lower average per cell (13.25 chiasmata) for 20 cells, and the proportion of bivalents with three chiasmata is definitely lower than in either *A. brevis* or *A. strigosa*. The unpaired chromosomes noted in *A. brevis* and *A. strigosa* were the result of anaphase separation of previously paired homologous chromosomes. As anaphase separation was not evident in any of the cells of *A. Wiestii*, the latter material was fixed at an obviously slightly earlier stage, and the difference between *A. Wiestii* and the other species is thereby emphasized.

Testing the independence of different species in respect to chiasma formation (Table VI), it is clear that the difference between *A. Wiestii* and *A. brevis* or *A. strigosa* is statistically significant while that between *A. brevis* and *A. strigosa* is not. Figs. 1, 2 and 3 show the chromosome complements of these three diploid species.



FIGS. 1–5. *Metaphase stage of first maturation division. Figures beside bivalents indicate numbers of chiasmata. Fig. 1. A. strigosa Schreb.; Fig. 2. A. brevis Roth.; Fig. 3. A. Wiestii Steudel.; Fig. 4. A. barbata Pott.; Fig. 5. A. abyssinica Hochst. ("naine"). Magnification* × *1300.*

TABLE VI

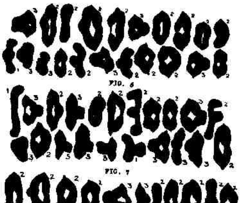
HOMOGENEITY OF SPECIES IN RESPECT TO CHIASMA FORMATION, χ^2 TEST

Species	Chiasmata							
	0	1	2	3	Total bivalents	χ^2	D.F.	Limit for significance (χ^2 for $p = .05$)
A. Wiestii *A. brevis*	0 1	22 14	111 83	7 42	140 140	31	3	7.8
A. strigosa *A. brevis*	1 1	15 14	84 83	40 42	140 140	.09	3	7.8
A. Wiestii *A. strigosa*	0 1	22 15	111 84	7 40	140 140	29	3	7.8
A. abyssinica ("naine") *A. barbata*	2 0	42 10	116 68	22 20	182 98	10	3	7.8
A. abyssinica ("naine") *A. abyssinica* (gray)	2 0	42 29	116 130	22 23	182 182	5.1	3	7.8
A. barbata *A. abyssinica* (gray)	0 0	10 29	68 130	20 23	98 182	4.0	2	5.9
A. sativa *A. sterilis* (6)	0 0	5 14	93 91	49 42	147 147	4.8	2	5.9
A. sterilis (6) *A. sterilis* (5)	0 0	14 15	91 108	42 66	147 189	1.5	2	5.9
A. sterilis (5) *A. sativa*	0 0	15 5	108 93	66 49	189 147	3.1	2	5.9
A. barbata *A. strigosa*	0 1	10 15	68 84	20 40	98 140	3.1	3	7.8
A. Wiestii *A. abyssinica* (gray)	0 0	22 29	111 130	7 23	140 182	5.6	2	5.9
A. Wiestii *A. barbata*	0 0	22 10	111 68	7 20	140 98	14.1	2	5.9
6*x* hybrid *A. sterilis* (6)	0 0	23 14	129 91	58 42	210 147	.20	2	5.9
6*x* hybrid *A. sativa*	0 0	23 5	129 93	58 49	210 147	7.2	2	5.9

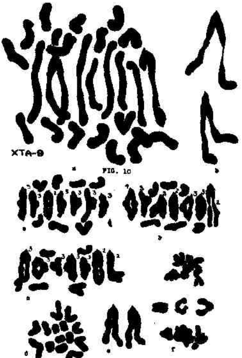
(*b*) *Tetraploid Species*

Chiasma frequencies for tetraploid species are recorded in Table III. Applying the χ^2 test to the totals from Table III, the difference between chiasma frequency in *A. barbata* and *A. abyssinica* "naine" is found to be statistically significant. The difference between the two forms of *A. abyssinica* and that between *A. barbata* and *A. abyssinica* (gray) are not statistically significant. As the number of tetraploid cells is low, and as the mean number of chiasmata per cell appears to differ, a comparison between mean chiasma frequencies of the different species is also made. In order to increase the

number of observations upon which the estimates of the standard errors are based, both diploid and tetraploid figures are included in an analysis of variance (6). The standard deviation thus calculated is 1.075. Table VII shows significant differences in mean chiasma frequency per cell.



FIGS. 6–9. *First meiotic division. Fig. 6. A. sterilis L (parent of 5x hybrid); Fig. 7. A. sterilis L (parent of 6x hybrid); Fig. 8. A. sativa L var. Radnorshire Sprig; Fig. 9. 6x hybrid: a. 21 bivalents of one spore mother cell; b. quadrivalent configurations. Magnification × 1300.*



FIGS. 10 and 11. *First meiotic division. Fig. 10. 5x hybrid: a. 9 bivalents, 17 univalents of one cell; b. trivalent configurations. Fig. 11. 3x hybrid: a. 7 univalents, 7 bivalents; b. 6 univalents, 6 bivalents, 1 trivalent; c. 5 univalents, 5 bivalents, 2 trivalents; d. polar view showing peripheral position of the 7 univalents; e. typical trivalents with one chromosome showing two chiasmata in one arm, and one chiasma in the other arm; f. equational division of lagging univalents. Magnification × 1300.*

TABLE VII

SIGNIFICANT DIFFERENCES IN MEAN CHIASMA FREQUENCY PER CELL IN VARIOUS SPECIES OF *Avena*

Species	Mean number chiasmata per cell	*n*	Standard error of mean	Mean difference	Standard error, $M_1 - M_2$	Necessary difference between means†
(1)* *A. strigosa*	15.15	20	.24038	1.9 (1–2)	.3399	.66620
(2) *A. Wiestii*	13.25	20	.24038	.15 (1–3)	.3399	.66620
(3) *A. brevis*	15.30	20	.24038	2.05 (2–3)	.3399	.66620
(4) *A. barbata*	29.42	7	.40627	3.27 (4–5)	.5040	.98784
(5) *A. abyssinica* "naine"	26.15	13	.29816	1.88 (4–6)	.5040	.98784
(6) *A. abyssinica* (gray)	27.54	13	.29816	1.39 (5–6)	.4217	.82653

* *The meaning of these numbers will be clear from Column 5, in which pairs of "mean numbers Xta per cell" obtained from Column 2, and identified by the numbers in brackets (obtained from Column 1), are subtracted from each other, and the results tabulated.*

† *Necessary difference = standard error of mean difference* × $t_{(p=.05,\ n=39)}$

The only mean difference that is insignificant is that between *A. strigosa* and *A. brevis.*

Fig. 4 illustrates the chromosome complement of *A. barbata*, Fig. 5 that of *A. abyssinica* "naine". In the latter there are two unpaired chromosomes.

(c) Hexaploid Species

Chiasma frequencies of hexaploid species are given in Table V. The χ^2 test (Table VI) fails to reveal any significant differences between chiasma frequencies of the species investigated. Figs. 6, 7 and 8 show metaphase stages of the hexaploid forms.

(d) 6x Hybrid

There is little difference between chiasma formation in the hexaploid hybrid (Fig. 9*a*) and in its low-frequency parent, *A. sterilis*. The difference between the hybrid and its other parent, *A. sativa* var. Radnorshire Sprig, is statistically significant. (See Table VI.) Multivalent configurations were seen frequently. Two out of ten cells selected at random showed quadrivalent complexes (Fig. 9*b*).

(e) 5x Hybrid

Chiasma frequency in the hybrid is markedly lower than in its parents, *A. abyssinica* "naine" and *A. sterilis*. With complete allosynapsis, and no autosynapsis, seven univalent chromosomes would occur at the first maturation division. In the hybrid, the number of univalent chromosomes varied from 13 to 23 (Fig. 10*a*). In 30 cells selected at random, six trivalent configurations were seen (Fig. 10*b*): no quadrivalents were found.

(f) 3x Hybrid

Pairing was closer in the triploid hybrid, *A. barbata* × *A. strigosa*, than in the pentaploid hybrid. In spite of the fact that at least five of the seven supernumerary chromosomes of *A. barbata* remained unpaired in all of the observed cells (Figs. 11*a*, *b*, *c*, *d*), the average number of chiasmata per bivalent of the hybrid was quite high, being 1.72, while those of its parents are 2.10 and 2.16 respectively. Two of twelve cells showed multivalent configurations. One had one, the other two, trivalents.

Discussion

In most organisms, the pairing of chromosomes at metaphase of the first division of meiosis is conditioned by chiasma formation which takes place between paired homologous chromosomes during pachytene. The zygotene synapsis depends primarily upon the degree of homology which exists between similar paternal and maternal chromosomes, but the following factors may have an effect, more or less marked, either upon prophase pairing or upon subsequent chiasma formation:

(*a*) While pairing is primarily conditioned by homology, it also depends upon the arrangement of homologous parts, and an inversion may interfere with pairing in a heterozygous individual while it causes no external varietal or specific differentiation between the parents.

(*b*) The amount of pairing may vary at different stages in anther development. Hollingshead (7) has shown that when the "general stage" of the anther was late, that is, when most of the cells of the anther had completed division, the proportion of univalents at meiosis was higher than normal in a number of Marquis wheat plants.

(*c*) It is possible that external conditions may influence pairing as Kihara has suggested (10).

(*d*) Competition in pairing may cause a reduction in chiasma frequency in polyploid forms.

(*e*) The pairing process is subject to genetical control. Pairing of homologous chromosomes may take place normally at early stages, yet through failure of chiasma formation there may, at metaphase, be no indication of this earlier association. This condition is seen in an asynaptic maize, where it is shown to be due to an "asynaptic gene" (Beadle (2)). Genetic factors may also interfere with the early association. The "*c*" chromosome missing from certain asynaptic 40-chromosome dwarf fatuoid oats and speltoid wheats apparently carries factors necessary for normal pairing (Huskins and Hearne (9)). Peto (14) investigated a number of hybrids between two different genera of the Gramineae, *Festuca* and *Lolium*. On back crossing a number of individuals of a given F_1 population to one parent, the progeny fell into markedly different classes with respect to chiasma frequency. This fact is attributed to segregation of genetical factors limiting chiasma formation.

It is very evident from these and similar observations that regularity in pairing depends not only upon homology, but also upon environmental and developmental influences, and upon a balanced condition of genes, or the presence of definite genes, that permit prophase pairing of similar chromosomes, and the maintenance up to anaphase of this pairing through chiasma formation.

Comparison Between Chiasma Frequencies of Different Species

Any significant difference in chiasma frequency of the oats species under consideration in this paper may conceivably be dependent upon one or all of the above mentioned factors. The chances of structural hybridity must, however, be small in the individuals of a self-fertilizing genus such as *Avena*, and the developmental effect was probably eliminated in these experiments. Fixations were made at what appeared to be comparable stages in anther development, that is when the majority of cells were at metaphase stage, and, as already noted, the various oats species, with the exception of *A. abyssinica* (gray), were grown simultaneously under similar environmental conditions in experimental plots. In so far, then, as the differences noted are probably due chiefly to differences in the genes which affect pairing and chiasma formation, we might expect some parallelism between similarity in chiasma formation and closeness of relationship. But the use of chiasma frequency as an indication of specific relationships is seriously limited by the fact that gene differences leading to wide variation in respect to this characteristic may occur within a pure species (Beadle (2), Huskins and Hearne

(9), Philp and Huskins (15), Armstrong and Huskins (1)). Hence a diffe ence in chiasma frequency between two species does not *necessarily* impl that the two species concerned are not closely related. It seems more likel however, that in a marked similarity of chiasma frequencies of two differer species we have evidence in favor of a close relationship. At most, we ma expect some parallelism between close relationship and similarity in chiasm formation.

We may review the present results in relation to the genealogical schem of Thellung (17) which is given in Table VIII in a slightly modified form.

TABLE VIII

GENEALOGICAL SCHEME OF THELLUNG (17)

Kulturformen	*A. byzantina* C. Koch	*A. nuda* L. ? *A. sativa* L.	*A. strigosa* Schreber (inkl. *A. brevis* Roth)	*A. abyssinica* Hochst.
Wildformen	1. *A. sterilis* L.	2. *A. fatua* L.	3. *A. barbata* Pott ex Link	3a. *A. Wiestii* Steudel
	§ 1. Biformes	§ 2. Conformes		

The species, arranged according to mean chiasma frequency per bivalent, give the following series: *A. abyssinica* "naine", 1.87; *A. Wiestii*, 1.89; *A. abyssinica* (gray), 1.96; *A. barbata*, 2.10; *A. strigosa*, 2.16; *A. brevis*, 2.18; *A. sterilis*, 2.19; *A. sterilis*, 2.26; *A. sativa* var. Radnorshire Sprig, 2.28. As the differences are slight, the variability within species wide, and the number of cells upon which the mean is based is small in some cases, one cannot attach much importance to these figures. However, the relative positions of the different species in the series are suggestive enough to warrant the application of statistical methods to the data available.

The homogeneity of species in respect to frequency of bivalents with zero, one, two, three, or four chiasmata is tested by means of χ^2. No significant difference is found between the three related species *A. brevis*, *A. strigosa* and *A. barbata*. *A. Wiestii*, which has a relatively low chiasma frequency, differs significantly from the other diploid species and from *A. barbata*, but does not differ significantly from the related species *A. abyssinica* (gray). So far, the results are consistent with the idea that similarity in chiasma formation may be correlated with closeness of relationship. However, the results within the tetraploid and hexaploid groups fail to reveal the expected parallelism.

Among the tetraploids, *A. barbata* and *A. abyssinica* (gray) show no significant difference although they fall into different subdivisions of Thellung's (17) table. There is a significant difference between *A. barbata* and *A. abyssinica* "naine", but as the latter had unpaired univalents in two out of thirteen cells investigated, and as it was slightly dwarf in habit, it is probably

an aberrant form, and *A. abyssinica* (gray) is the more typical representative of the species. The mean chiasma frequency per cell of *A. barbata* did differ significantly from both strains of *A. abyssinica*, but as the two strains also differed significantly from each other, it is obvious that intraspecific differences may be quite as significant as interspecific ones. *A. sativa* and *A. sterilis* fall into different sections of Thellung's (17) table, but the χ^2 test fails to reveal any significant difference between any of the hexaploid forms studied. It is possible that a concentration of genes favoring chiasma formation is responsible in these polyploids for a maximum effect, thus preventing cytological differentiation of species by means of chiasma studies, even though the species from which they arose may have shown significant differences in chiasma frequency. There is no evidence from these studies that chiasma frequency in the polyploid oats is reduced by competition in pairing.

Chiasma Frequencies in Triploid, Pentaploid and Hexaploid Hybrids

In the pure species every chromosome has its identical mate, but in the species hybrids a given chromosome may or may not have a homologous mate. The degree of homology may conceivably vary, and the closer the relationship between the parental forms, the greater the chance of homology between their chromosomes. But again the questions of environment, nature of the genes that affect pairing and chiasma formation, and, in addition, structural chromosome changes, are all complicating factors. A lack of pairing does not necessarily indicate a lack of homology, but close synapsis must indicate a considerable degree of homology between the chromosomes that pair. Chiasma count at metaphase is a less direct indication of homology than observations on synapsis, but it is the best criterion available for this material as it is not possible by present methods to study the whole chromosome complement critically at early stages.

In the triploid hybrid metaphase pairing was close. In all of the observed cells, there were at least seven bivalents, presumably seven chromosomes of *A. strigosa* paired with seven chromosomes of *A. barbata*. In half of the observed cells, however, more than 14, and usually 16, univalents were involved in chiasma formation. The average number of chiasma per bivalent in the hybrid, as calculated on the basis of 7.5 bivalents per cell, is 2.40. The average number in the parental strains is 2.19 and 2.16 respectively. The higher number in the hybrid is somewhat unexpected, but is perhaps due to different methods of fixation. It is at any rate evident that there exists a very considerable degree of homology between the chromosomes of *A. strigosa* and seven of the chromosomes of *A. barbata*. In addition to the pairing of seven chromosomes from each parent, one, two or three of the supernumerary chromosomes of the tetraploid parent entered at times into pairing relationships. Cells with eight bivalents were observed, and in the same hybrid Nishiyama (12) has found cells with nine bivalents. In 10 cells chosen at random, three trivalents were noted. Although no quadrivalents were observed in the material examined, Nishiyama (12) reports rare quadri-

valents in the hybrid he studied. He also found as many as three trivalents in one cell. Such behavior might be explained by the following scheme:

2x	4x	
a a	a a	a ab b
b b	b b	a ab b
c c	c c	c cv v
d d	d d	w wv v
e e	e e	x x
f f	f f	y y
g g	g g	z z

(i) Seven chromosomes of the diploid are homologous or very nearly homologous to seven tetraploid chromosomes.

(ii) Three supernumerary chromosomes of the tetraploid have no homologous or partially homologous mates among the diploid chromosomes.

(iii) Three supernumerary chromosomes are partially homologous to three chromosomes of the diploid. Hence the occurrence of as many as three trivalents in one cell.

(iv) Postulate duplications between two pairs of the supernumerary chromosomes, and autosynapsis as well as the appearance of the occasional quadrivalent configuration is accounted for.

Observations on the triploid hybrid lead to the conclusion, already suggested by comparative study of chiasma frequency of *A. barbata* and *A. strigosa*, that these two species are very closely related.

Pairing in the pentaploid hybrid as judged by chiasma formation, is looser than in the triploid hybrid discussed above. More than half the potential bivalents, that is 14 per cell, had no chiasmata, 44.5% had one, and 4% had two. The number of bivalents per cell varied from 5 to 11 (Fig. 10*a*). Nishiyama (12) in the pentaploid hybrid *A. barbata* × *A. fatua* found 2 to 11 bivalents, including 1 to 4 trivalents, and in *A. barbata* × *A. sterilis* 7 to 13 bivalents, including 0 to 4 trivalents. Emme's (5) results correspond with those of Nishiyama. In general then, crosses involving *A. barbata* or *A. abyssinica* and *A. sterilis* or *A. fatua* indicate either a lack of close relationship between the chromosomes of these tetraploid and hexaploid species, or the lack of genes favorable to synapsis or chiasma formation. The evidence is in favor of the former alternative, for our results with pure species suggest that a concentration of genes in polyploids is likely to result in the maximum chiasma formation between homologous chromosomes. It should be noted, however, that in this case the tetraploid parent was probably an aberrant form with chiasma formation lower than that normal to the species. Trivalents were seen in several of the pentaploid cells (Fig. 10*b*).

The hexaploid hybrid *A. sterilis* × *A. sativa* var. Radnorshire Sprig showed practically the same chiasma frequency as did its low frequency parent *A. sterilis*, but the χ^2 test indicates that the hybrid and *A. sativa* var. Radnorshire Sprig do differ significantly in respect to chiasma formation. Although *A. sativa* and *A. sterilis* are not commonly considered very closely related species, the cytological evidence of a difference between them is slight. Close pairing in the hybrid may have been due in some small measure to autosynapsis between partially homologous chromosomes present through polyploidy, although multivalents and univalents were seen in only about 20% of the cells. However, this cannot be established cytologically, as the origin of the paired chromosomes cannot be determined. The cytological study of species reveals no significant difference, so that what little evidence is afforded by these studies is based upon the difference between the hybrid and its parent, *A. sativa* var. Radnorshire Sprig. It may be noted that certain characters of the variety Radnorshire Sprig suggest affinities with *A. byzantina* C. Koch, a species associated with *A. sterilis* in the relationship schemes of various authors.

Nishiyama (12) found in his hexaploid hybrids sometimes one to four univalents in one cell. In the hexaploid hybrid under consideration in this paper, univalent chromosomes were not observed in cells whose full chromosome complement could be distinguished with certainty. A few cells, damaged by pressure, showed two univalents.

Chiasma formation in the three different hybrids is represented graphically in Fig. 12.

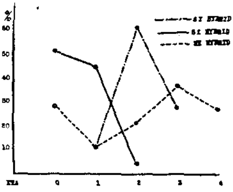


FIG. 12. *Percentage of bivalents with different numbers of chiasmata in the three hybrids studied.*

Conclusions and Summary

It is possible that chiasma frequency at metaphase is influenced by external and internal environment, competition in pairing in polyploids, genes affecting synapsis and chiasma formation, structural hybridity and the degree of homology between members of a bivalent.

The only direct indication of relationship between the oats species studied is homology of chromosomes as judged by pairing behavior in the hybrid. A similarity of genes that affect synapsis and chiasma formation may indicate relationship between pure species. The cytological determination of relationship is complicated by the remaining factors, and by the fact that intraspecific gene differences may be as great as interspecific ones.

A comparison of different species of *Avena* with respect to chiasma formation reveals significant differences between certain diploid species. The closely allied forms *Avena strigosa* and *A. brevis* are alike, but both differ significantly from *A. Wiestii*. The differences between the tetraploid forms studied are of questionable significance, those between the hexaploid forms insignificant. It is suggested that a concentration of genes favoring synapsis and chiasma

formation is responsible for the lack of significant differences between polyploid forms which are not closely related. The relationships between diploid and tetraploid species are also indicated by similarities in chiasma frequency. *A. brevis*, *A. strigosa* and *A. barbata* show similar chiasma formation, while *A. Wiestii* resembles *A. abyssinica* in this respect.

The hybrid investigations yield results in accord with those of previous workers. In the triploid and hexaploid hybrids, pairing is close, in the pentaploid hybrid relatively loose.

Close pairing in the triploid is considered an indication of close relationship between *A. strigosa* and *A. barbata*, a conclusion also supported by chiasma studies in the pure species. Low chiasma formation in the pentaploid hybrid *A. abyssinica* × *A. sterilis* suggests lack of homology between parental chromosomes. Chiasma formation in the hexaploid hybrid is high. While it may be that this results in part from autosynapsis, the cytological evidence fails to indicate anything more than a very slight non-homology between chromosomes of the parental species, *A. sativa* var. Radnorshire Sprig and *A. sterilis*.

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STUDIES IN THE VARIABILITY OF TUBERCLE BACILLI.

VIII. BCG.[1]

By G. B. Reed,[2] J. H. Orr[3] and Christine E. Rice[4]

Abstract

Five cultures of BCG have been shown to consist primarily or entirely of R type tubercle bacilli and to resemble R types from other sources. When received one culture contained a few S types which were isolated. The other four cultures appeared to consist entirely of R types. After a long series of cultures, particularly in slightly alkaline Proskauer and Beck's fluid, dissociation occurred and S types were isolated from two strains; no stable S types could be recovered from two additional strains.

The R, or ordinary BCG produced only localized infections, the S forms produced fatal progressive tuberculosis in guinea pigs. The degree of tissue involvement in fatal cases was generally less than with typical virulent tubercle bacilli.

By means of complement fixation reactions it was shown that the S form recovered from BCG cultures, like typical S virulent mammalian tubercle bacilli, contains a specific antigenic substance which is lacking in the ordinary BCG and other R forms of the species. Animals immunized with S organisms are shown to develop a specific S antibody which is not present in the serum of animals treated with R organisms or the ordinary form of BCG. It is suggested that this lack of antigenic substance in the BCG militates against its effectiveness as an immunizing agent.

1. Introduction

The greater part of the now voluminous literature on the stability and avirulence of the bacillus of Calmette and Guérin is in agreement with the view of Calmette (8) and his associates that this strain of the tubercle bacillus is a stable, true-breeding, avirulent or nearly avirulent organism. There is almost the same unanimity of opinion that vaccination with living cultures of this organism imparts a degree of resistance against virulent tubercle bacilli. In the hands of a few observers, especially Petroff (17–21), Begbie (4), Watson (37, 38), Sasano and Medlar (32), Dreyer and Vollum (10), Seiffert (33), the culture has exhibited sufficient variability that virulent types have been isolated, or progressive disease produced in animals. In his more recent work Watson (38) has found that the virulence, rarely manifested in the earlier cultures, has completely disappeared in their descendants since 1928–29.

It has now been amply demonstrated that tubercle bacilli and related acid-fast species of bacteria may exhibit variation similar to that shown by other bacterial species. Petroff, Branch and Steenken (19, 20) and later Petroff and Steenken (21) described two colony forms exhibited by several cultures of human, bovine and avian tubercle bacilli. They found that virulence was generally associated with one colony type, the S, and low virulence generally associated with another type, the R. This was later confirmed by

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Begbie (4), in earlier papers in this series, by Reed and Rice (26, 27), Reed (24), by Kahn and Schwarzkopf (13, 14) and others. In the earlier papers from this laboratory it was shown that in a considerable series of cultures of tubercle and other acid-fast species, recently isolated virulent cultures, or cultures which had long maintained high virulence, grew in S colonies or exhibited a preponderance of S colonies, grew in a characteristic habit in fluids and exhibited acid agglutination only in strongly acid buffer solutions; avirulent cultures or cultures with a history of loss of virulence grew in R colonies on solid media, in a characteristic habit in fluid media and exhibited acid agglutination when suspended in relatively less acid buffer solutions. Cooper (9) found that S and R forms from the same strain differ in chemical composition; Kahn and Schwarzkopf (13) demonstrated differences in electrophoretic potentials and Reed and Gardiner (25) found differences in electrophoretic potential, acid agglutination and the isoelectric points of suspensions of S and R types. An apparently more significant difference between the R and S types was demonstrated in the writers' earlier papers, Rice (29), Rice and Reed (31), Reed, Rice and Gardiner (28); it was shown that the S types of tubercle bacilli contain an antigenic substance which is lacking in the R types.

In the original observations of Petroff and his associates (17–20) it was found that BCG cultures produced primarily R colonies. In certain of these cultures, however, they were able to demonstrate a small proportion of S colonies which were isolated and found to be similar in colony structure and in virulence to other S mammalian tubercle bacilli. This was confirmed by Begbie (4) and more recently by Seiffert (33). On the other hand several observers, including Birkhaug (2) who has reviewed the earlier observations, have isolated S-like colonies but found them to be as avirulent as the original BCG. Others contend that the S-like forms isolated by Petroff are human tubercle bacilli and, as BCG was developed from a bovine strain, regard the S forms in his cultures as contaminations. This appears to have been Calmette's (8) opinion, but it is not shared by those most familiar with Petroff's work.

Any differentiation of strains of tubercle bacilli on the basis of colony structure is at present very difficult owing to the fact that no two workers appear to be in agreement as to the structure of S and R colonies. Seiffert (33), who has probably made the most exhaustive study, describes several sub-groups of both S and R types recovered from BCG cultures. Our own results with BCG indicated in more detail below, are, in part, in agreement. What we regard as R types, Figs. 1 and 2, have been regularly recovered from BCG cultures. These colonies appear to be the same as Seiffert's (33) Hohe Haufchenform and the R forms described by Petroff (17–21) and others who have worked with this culture. In the course of long cultivation of cultures of these R types most of the R sub-types of Seiffert have been observed but we have not succeeded in stabilizing them to a sufficient degree to obtain true breeding cultures. As noted in a later section, one of Seiffert's R types in particular so closely resembles S types that at times there has been considerable confusion.

The S colonies which we have obtained from two of the five cultures of BCG studied, Figs. 4, 5, 6 and 7, as just noted, resemble S colonies obtained from other mammalian strains of tubercle bacilli (Reed and Rice (27)). They are apparently the same as Seiffert's (33), Trockene Halbkugelform or Trockene Schiebform and the S forms described by Petroff (17, 18) and Begbie (4) as recovered from BCG. Fig. 9 indicates one S colony from a bovine type culture, Vallée strain, and Fig. 10 one S colony from a human type culture, a strain recently isolated from sputum, and grown under the same conditions as the S forms recovered from the BCG cultures. As in the case of the R forms we have from time to time observed the other S forms described by Seiffert but have not succeeded in isolating them in stable form. Birkhaug (3) on the other hand describes an "S form" recovered from BCG as "hemispherical, of a creamy consistency and smooth surface." This does not appear to agree with any other description of S forms of mammalian tubercle bacilli except possibly Seiffert's (33) Feuchte Halbkugelform. It is not apparent whether this is the "S type" of BCG which Birkhaug described in an earlier paper (2) as avirulent for guinea pigs.

Several investigators, without including a study of dissociation from the point of colony structure, have reported a general increase in the virulence of BCG cultures (Watson (37, 38)). In a similar manner Sasano and Medlar (32) found that serial cultures in Sauton's fluid containing normal rabbit serum and Dreyer and Vollum (10) found that cultures in broth under semi-anaerobic conditions resulted in greatly enhanced virulence. Boquet (7) however was unable to confirm either of the last two results.

In the present study some five strains regarded as BCG have been examined. As noted in the following sections they were obtained from Dr. Petroff of Trudeau, Dr. Watson of Ottawa, Dr. Rankin of Alberta, Dr. Parizeau of Montreal and M. Nègre of Professor Calmette's laboratory at the Pasteur Institute. These cultures were received and have been examined over a period of some five years. They have not all been subject to the same treatment or examined for the same periods so that the results with the several cultures cannot be directly compared. The general procedure has been to start from the cultures as received, a series in fluid media, generally Proskauer and Beck's synthetic medium and to make a number of plate cultures on gentian-violet egg media and generally also on Petragnani's egg-potato-malachite-green medium. In most instances the fluid cultures have been carried by serial transfers through many cultural generations with side cultures to solid media from time to time to determine the colony form and with frequent guinea pig inoculations to test the virulence.

2. Results with the Trudeau Strain of BCG

Dr. Petroff was kind enough to send us a culture of BCG, presumably one of those used in his study of dissociation (17–21). The primary series of plates on gentian-violet egg media made from this culture developed a majority of R colonies. These were similar in structure to the R types in all five strains

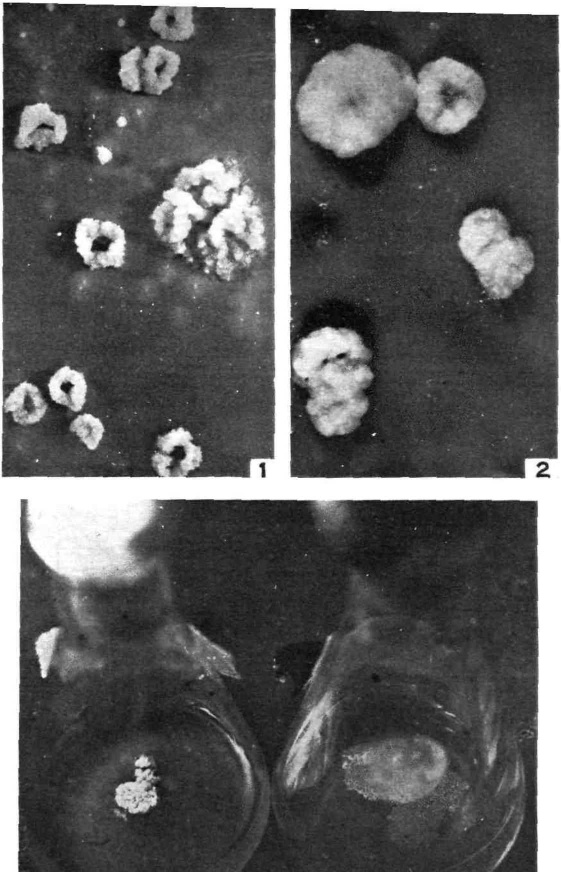

PLATE I. BCG COLONIES: FIGS. 1 AND 2. *Characteristic R colonies from the Ottawa and Alberta cultures.* FIG. 3. *Two cultures of the Ottawa strain on Proskauer and Beck's fluid media. At the left—a characteristic R BCG growth; at the right—an R-like mass of growth with a larger area of spreading veil from which an S type was eventually isolated.*

PLATE II

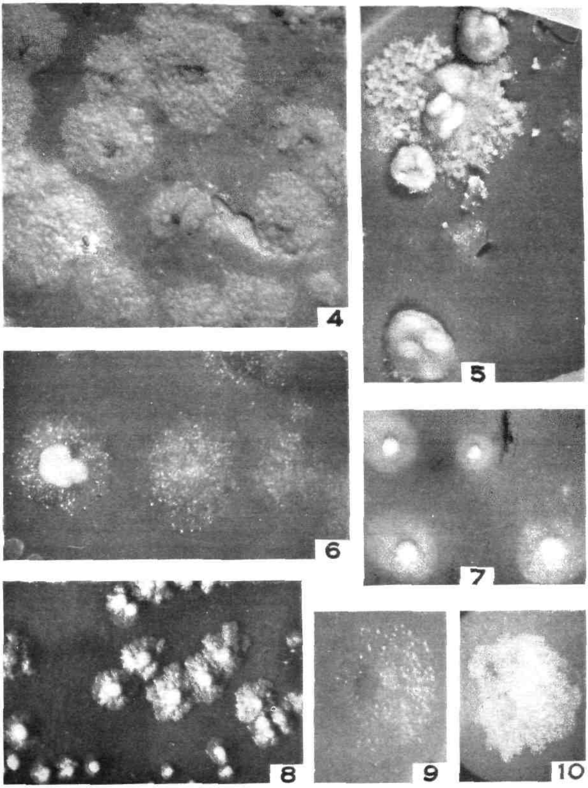

PLATE II. BCG COLONIES. FIG. 4. *S colonies isolated from the Trudeau culture.* FIG. 5. *R and an intermediate or S colony spreading out from an R colony, from the Alberta culture.* FIG. 6. *At the left—an R colony with peripheral zone of S growth; right—2 S colonies.* FIG. 7. *S colonies isolated from the Ottawa culture.* FIG. 8. *R colonies from the Montreal culture, resembling Seiffert's Feuchte Körnchenform, frequently confused, when young, with S.* FIG. 9. *S colony bovine tubercle bacilli, Vallée strain, grown under the same conditions as the BCG.* FIG. 10. *S colony human tubercle bacilli recently isolated from sputum of an active case of pulmonary disease and grown under the same conditions as the BCG.*

examined and appear to be identical with those figured by Petroff (17–21) and Begbie (4), and closely resemble R colonies previously described from other strains of tubercle bacilli (Reed and Rice (26, 27)). (Figs. 1 and 2.) The primary plates, however, also developed a considerable number of very definite S colonies.

The most S-like were fished and replated; after one to six platings from single colony isolations, strains were obtained which produced only S colonies for several serial cultures on gentian-violet egg media. These S colonies (Fig. 4), with their much flattened conical structure, thin spreading and irregular outline and finely granular surface resemble S colonies from many other cultures of mammalian tubercle bacilli (Reed and Rice (27)), and contrast sharply with the R-like colonies of the usual BCG culture (Figs. 1 and 2). In fluids such as Sauton's or Proskauer and Beck's media, the more or less stabilized S first grew as a thin spreading veil which later became thickened and folded in constrast to the ordinary BCG which grows as a thick granular pellicle readily separating into island-like masses. The S forms as shown in section 8 contained the S specific antigenic substance absent in the R type or ordinary BCG.

Doses of one to five milligrams of the S culture injected intracardially or intraperitoneally into guinea pigs produced generalized tuberculosis and death in three to six weeks.

It seems evident, therefore, that this culture when received consisted of two types, distinguishable by colony structure, antigenic content and virulence.

3. Results with the Ottawa Strain of BCG

Dr. Watson, of the Canadian Department of Agriculture very kindly provided us with a culture of BCG marked "80B", evidently one of those used in his study of BCG reactions of calves (37, 38).

In sharp contrast with the Trudeau BCG the initial series of cultures from this strain developed only the R type of colony (Figs. 1 and 2). It is possible that more plates might have demonstrated some variability but it could not be found among some 50,000 to 100,000 colonies compared in the original series and, as shown in more detail in Table I, several guinea pigs inoculated with five-milligram doses failed to develop generalized tuberculosis in three to six months.

Proskauer and Beck's fluid media made slightly alkaline, pH 7.4 to 8.0 in different lots, was inoculated from the original culture and carried through some 30 serial cultures over a period of three years, with numerous side cultures in this or other fluid media and to plates for colony structure studies. Animal inoculations were made direct from many of the Proskauer and Beck serial cultures or from the side series.

In the serial cultures in fluid media advantage was taken of the difference in the habit of growth of S and R forms. As described by Petroff (17, 18),

and discussed in some detail in an earlier paper by Reed and Rice (27) human and bovine tubercle bacilli of the R type grow on the surface of many fluid media as a thick granular pellicle readily separating into island-like masses during the first two to three weeks of growth. In conspicuous contrast, especially in very young cultures, the S type tends to grow in a continuous, flat, spreading, veil-like pellicle. Accordingly any tendency to veil-like growth was taken as suggestive of the appearance of an S variant. This tendency was generally most readily observed where rather large volumes of culture fluid were used (100 to 300 cc. amounts in 250 cc. or litre Erlenmeyer flasks). These veil-like areas appeared as marginal outgrowths from granular masses or as continuous connections between islands of granular pellicle (Fig. 3). In the serial transfers to Proskauer and Beck's fluid, as well as to the side cultures on plates, inoculum was always taken from the more definite veil-like growths.

The most important features of this series of cultures are summarized in Table I. It will be observed that the first 14 serial cultures on the Proskauer and Beck's medium resulted in the characteristic heavy, granular, island type of pellicle; all the plates developed R colonies characteristic of typical BCG. The small number of guinea pigs inoculated developed only the usual localized BCG type of reaction. It is quite possible that with large numbers of guinea pigs, as used by Uhlenhuth and Seiffert (35) or if the animals had been kept under observation for long periods as in certain of Watson's (37, 38) experiments, some generalized cases would have appeared. But within the limitations of the experimental procedure this culture behaved for these 14 cultural generations, approximately two years, as a characteristic BCG.

As noted in Table I, the cultures from the ninth serial transfer developed some traces of the thin spreading veil which was used for inoculum for the subsequent cultures. Although the initial plates from this veil failed to yield colonies different from the heavier pellicle, the fifteenth serial fluid culture when plated developed a few apparently S colonies. Successive platings from the veil resulted in increase in this type of growth at the expense of the heavy granular or island habit, and by the twenty-eighth or twenty-ninth culture plates, produced exclusively the S type of colonies. At the same time that a preponderance of the colonies developed in the S form, doses of from one to five milligrams injected intraperitoneally into guinea pigs resulted in generalized tuberculosis and death in three to eight weeks.

S colonies were also isolated from the earlier plates on which they appeared and, as in the case of the Trudeau culture, after several replatings from single S colony isolations S strains were established similar to those obtained by veil transfers in the serial fluid cultures (Figs. 6 and 7).

The S forms, obtained by either procedure, as in the former case, were distinguished from the R or ordinary BCG by acid agglutination, by antigenic structure (section 8) as well as by the differences in colony structure and virulence.

TABLE I

COLONY STRUCTURE AND VIRULENCE OF THE OTTAWA BCG DURING THE COURSE OF SERIAL CULTURES ON PROSKAUER AND BECK'S ALKALINE FLUID. VEIL WAS USED AS FAR AS POSSIBLE FOR INOCULUM

No. of generations	Special cultures in P. and B. media	Colony structure, on solid media	Guinea pig reaction
1	Island type	All R	6 pigs, 5 mg. intrap. No generalized Tb. after 3 mo.
2 to 8	Island type	All R	
9	Islands, trace veil	All R	6 pigs, 5 mg. intrap. 2 died 6 and 20 days with extensive Tb. No generalized Tb. after 3 mo. in remaining 4.
10	Islands, trace veil	All R	
11	Islands, trace veil	All R	6 pigs, 5 mg. intrap. No generalized Tb. after 3 mo.
12 to 13	Islands, trace veil	All R	
14	Islands, trace veil	All R	6 pigs, 5 mg. intrap. No generalized Tb. after 1 to 6 mo.
15	Islands, trace veil	Mostly R, few S	
16	Islands, trace veil		
17	Islands, trace veil	Mostly R, few S	
18	Islands, trace veil	Mostly R, few S	6 pigs, 5 mg. intrap. No generalized Tb. after 1 to 5 mo.
19	Islands and veil	Mostly R, few S	
20	Islands and veil	R and S	
21	Islands and veil	Mostly S, few R	4 pigs, 5 mg. intrap. 1 died intercurrent. No generalized Tb. in 2 at 50 and 98 days. Tb. of glands, spleen, liver, in 1 at 8 mo.
22	Islands and veil	Mostly S, few R	2 pigs, 5 mg. intrap. Generalized Tb. at 80 and 74 days.
23	Islands and veil	Mostly S, few R	4 pigs, 5 mg. intrap. 1 died intercurrent. 3 died generalized Tb. at 101, 30, 106 days.
24 and 25	Islands and veil	Mostly S, few R	
26	Veil, few islands	Mostly S, few R	6 pigs, 5 mg. intrap. 1 died intercurrent. 5 died generalized Tb. at 60, 46, 21, 73, 63 days.
			3 pigs and 2 rabbits inoc. from spleen of latter pig—intrap. 3 pigs died generalized Tb. at 6, 16, 18 days. 2 rabbits died generalized Tb. 18, 24 days.
27	Veil, very few islands	Mostly S, few R	
28	All veil	All S	
29	All veil	All S	
30	All veil	All S	6 pigs, 1–3 mg intrap. 2 died intercurrent. 4 died generalized Tb. at 29, 20, 38, 52 days.

It is perhaps surprising that this culture should have initially and for two years of serial transfers exhibited only R colonies and the usual BCG type of virulence, in view of the fact that Watson (37, 38) reported progressive tuberculosis in certain of his experimental animals inoculated with the culture. In our earlier studies on the dissociation of R type tubercle bacilli it was

repeatedly observed, Reed and Rice (27), Reed (24), that the S organisms first recovered from dissociating cultures were extremely unstable, and readily reverted to the R form. The same condition appears to be even more characteristic of the S forms recovered from this culture. In many instances cultures which behaved for one to several cultural generations as an S type again reverted to the R form. It is therefore conceivable that this particular culture when used by Watson for his animal inoculations contained S elements, but of an unstable character. In any case it is evident that the culture when received, and for more than a year of serial transfers, behaved like the many descriptions of BCG. It then gradually developed more and more S elements and increased in virulence for guinea pigs. This appears to be an ordinary instance of R to S bacterial variation as observed in many other species.

4. Results with the Alberta Strain of BCG

Dean Rankin, University of Alberta, was good enough to send us a culture of BCG, evidently the culture used in his extensive studies on the vaccination of calves (22, 23). As in the case of the Ottawa strain this culture, when received from Dr. Rankin, November, 1930, yielded on the primary plates only R types of colonies, and a small group of guinea pigs inoculated from the primary cultures developed only the typical localized BCG infection. As in the former case, this strain has been carried in serial cultures in Proskauer and Beck's slightly alkaline fluid. Where veil or any suggestion of veil-like pellicle appeared it was used for inoculum. Results from the more significant part of the series are summarized in Table II. As indicated there, in the initial culture on Proskauer and Beck's fluid, some traces of veil-like pellicle appeared, yet plates from the first five serial cultures failed to yield any definite S colonies, and guinea pigs injected with five-milligram doses developed only localized tuberculosis. The sixth to the tenth serial culture developed slightly more veil-like pellicle, and plate cultures showed more S-like colonies, yet five-milligram doses in guinea pigs failed to produce any rapidly developing or generalized tuberculosis. The eleventh serial culture did produce generalized infection in four guinea pigs, and plates from this culture developed a small proportion of characteristic S colonies. Several of these were fished and serial cultures carried on from single S colony isolations on gentian-violet egg media. As noted in Table II the third and fourth cultures of the latter series were largely or entirely S in colony structure (Fig. 5), and doses of five to one-tenth milligrams injected into guinea pigs resulted in rapidly progressive tuberculosis.

As in the former instances the establishment of stable S cultures was only effected after many serial transfers from single S colonies. Many S-like colonies when isolated failed to produce S progeny; many produced mixtures of types, but persistent selection yielded S cultures similar to those isolated from the other strains. As in other instances these S forms have been distinguished by antigenic structure (section 8) as well as by colony structure and virulence.

TABLE II

Colony structure and virulence of the Alberta strain of BCG during the course of serial cultures in Proskauer and Beck's fluid, and single colony isolation series on solid media

No. of generations	Serial cultures in P. and B. media	Colony structure	Guinea pig reaction
Serial cultures in Proskauer and Beck's:—			
1	Islands, trace veil	R only	6 pigs, 5 mg. intrap. killed after 3 mo. No generalized Tb.
2	Islands, trace veil	R only	
3	Islands, trace veil	R only	
4	Islands, trace veil	R only	6 pigs, 5 mg. intrap. 2 died intercurrent 38, 42 days. 4 killed 200 days no generalized Tb.
5	Islands, trace veil	R and few S-like	2 pigs, 5 mg. intrap. 2 died interc. at 60 and 70 days, no generalized Tb.
6 to 10	Islands, trace veil	R and few S-like	
11	Islands and veil	R and S-like	4 pigs, 5 mg. intrap. generalized Tb. at 90 days.
Serial cultures from single colony isolations beginning from the 11th Proskauer and Beck culture:—			
12		R and S	
13		Mostly S, few R	
14		Mostly S, few R	3 pigs, 5 mg. intrap. died with generalized Tb. at 28, 77, 83 days. 3 pigs inoculated from latter, died with generalized Tb. at 41, 66, 69 days.
15		Mostly or entirely S	2 pigs, 1 mg. intrap. died with generalized Tb. at 41, 60 days. 2 pigs 0.5 mg. intrap. died with generalized Tb. at 70 and 134 days. 2 pigs, 1 mg. intrap. died with generalized Tb. at 138, 175 days.

It seems apparent then that the Alberta culture when received behaved like a characteristic BCG or R form and that the changes just recorded represent another instance of R to S variation.

5. Montreal Strain of BCG

In December, 1931, Dr. Parizeau, Vice Dean, University of Montreal, was kind enough to send us a culture of BCG which he had received from the Pasteur Institute in 1928 and "reproduced every three weeks according to Professor Calmette's technique."

A large series of plates of gentian-violet egg media inoculated as soon as the culture was received, exhibited only R type colonies. Cultures were then started in Proskauer and Beck's fluid and carried through 27 serial transfers with the results indicated in Table III.

TABLE III

COLONY STRUCTURE AND VIRULENCE OF THE MONTREAL STRAIN OF BCG DURING THE COURSE OF SERIAL CULTURES ON PROSKAUER AND BECK'S FLUID MEDIA

No. of generations	Serial cultures in P. and B. media	Colony structure	Guinea pig reaction
1	Islands, trace veil		
2	Islands, trace veil	R, very few S?	2 pigs, 5 mg. intrap. Generalized Tb. yielding cultures—R and S. 2 pigs, intrap. from S? colonies of first, extensive Tb. yielding cultures—R and S. 4 pigs, intrap. from S? colonies of second—no Tb. after 3 mo.
3	Islands, trace veil	R, very few S	2 pigs, 5 mg. intrap. No Tb. after 3 mo.
4 to 6	Islands, trace veil		
7	Islands, trace veil	R, very few S	3 pigs, 5 mg. intrap. No reaction after 3 mo.
8 to 12	Islands, trace veil		
13	Islands, trace veil	R, very few S	4 pigs, 5 mg. intrap. Extensive Tb. yielding cultures—R and S? 2 pigs from S colonies of first—no Tb. after 11 mo.
14 to 26	Islands, trace veil		
27	Islands, trace veil	R and very few S?	6 pigs, 5 mg. intrap. Localized Tb. only after 3 mo.

From the first generation in Proskauer and Beck's medium this culture produced a small proportion of veil-like growth in contrast to the more massive R or island type of pellicle but, as indicated in Table III, notwithstanding 26 serial transfers from the veil-like structure, over a period of two years, the growth habit remained essentially unchanged. This is a contrast to the two previously described strains.

Subcultures from the veil-like growth to solid media always gave a preponderance of R type colonies and a very small proportion of S-like colonies. In many instances the S-like colonies were cultivated serially from single colony transfers for many generations. Much confusion was experienced from the development of colonies which at first appeared characteristically S-like but on further growth developed thicker margins and a much more irregular surface (Fig. 8), to resemble Seiffert's (33) "Flache Körnchenform" —a type which is avirulent and definitely not S. In no instance was a true breeding and characteristic S type obtained.

The veil-like growth obtained from the second generation on Proskauer and Beck's fluid, injected intraperitoneally in five-milligram doses into two guinea pigs, caused generalized tuberculosis with extensive lesions in the omentum, spleen and liver, and death in 30 and 87 days. Cultures from the tuberculous lesions in these animals exhibited a majority of definite R colonies and a small proportion of S-like colonies. Two guinea pigs injected with five-mg. doses of these S-like growths developed extensive tuberculosis. Cultures

from the lesions in these animals also exhibited a majority of R colonies with a few apparently S forms though they were less characteristic. Four guinea pigs injected intraperitoneally with five-milligram doses of these atypical forms exhibited only localized tuberculosis in the omentum. Essentially the same reaction was produced by inoculating guinea pigs with veil from the thirteenth serial transfer, while guinea pigs inoculated from the twenty-seventh serial transfer in Proskauer and Beck's fluid developed only characteristic localized BCG reactions.

It seems apparent therefore that although this culture exhibited some tendency to variation and did gain enough virulence to produce generalized or extensive tuberculosis in two lots of guinea pigs, the period of study was too short, or the methods followed were not successful in separating a stable true-breeding S form.

6. Paris Strain of BCG

In November, 1929, Dr. Nègre of the Pasteur Institute was kind enough to provide us with a culture of BCG. A series of plates on solid media made from the primary culture developed only R colonies. The first eight serial cultures in Proskauer and Beck's fluid, and in the same medium containing 10% normal rabbit's blood, showed no tendency to vary from the typical, granular, island type of pellicle. Several small series of guinea pigs inoculated with five-milligram doses developed only localized reactions. The eighth to the fifteenth serial cultures exhibited traces of veil-like growth and plates from this veil developed a very small proportion of S-like colonies. In every instance, however, one to three subcultures of the S-like colonies resulted in reversion to typical R forms.

A longer series of observations would possibly have revealed more definite variation but it appears evident that this culture, like the Montreal strain, consists of more definitely fixed R types than the first three strains of BCG examined.

7. Pathogenicity of S Forms Recovered from BCG

As noted in the previous sections, definite S forms recovered from BCG cultures have produced generalized tuberculosis and death in guinea pigs and rabbits. All colonies with S-like form have not proved to be virulent. This has been especially true of the first S colonies isolated from cultures previously all R, and as already noted and listed in Tables I to III, many such supposed S colonies when subcultured have yielded only R progeny. The structure of the colony can only be taken as one feature of variation with which altered virulence may or may not be associated. The observed results might, on the other hand, be interpreted as evidence that slight variation in the organisms, too transitory to persist in subcultures or in inoculated animals, are registered in colony structure. At the same time established S colony types breed true to type in serial cultures and produce progressive disease in animals on serial passage.

With such established S types from the BCG cultures, intraperitoneal doses in guinea pigs of 0.1 to 5 mg. have resulted in progressive disease and death. But this progressive and fatal tuberculosis differs more or less conspicuously from the animal response to the usual virulent bovine and human strains of tubercle bacilli, in that massive involvement of many tissues does not occur. Following intraperitoneal inoculation of guinea pigs, the lesions have been most evident in the omentum, spleen, epididymis and liver, in the order named. Intracardial inoculations have always developed more widespread infections involving particularly the lungs, but the involvement of tissue has generally been distinctly less than is ordinarily the case with fatal generalized tuberculosis.

Guinea pig No. 587, for example, was given intraperitoneally two milligrams of a supposed S type recovered from the Ottawa culture of BCG. The animal died 63 days later. Post-mortem examination showed generalized thickening of the omentum with many nodular swellings which, on section, discharged creamy caseous material containing very large numbers of acid-fast bacteria presenting all the characteristics of tubercle bacilli. The spleen showed considerable enlargement and several gray to yellow nodules which, as in the case of the omental swellings, proved on section to contain caseous material and acid-fast bacilli. The liver presented a similar appearance though the nodules were much more scattered but here again acid-fast organisms appeared in smears from the nodules. The epididymis appeared reddish-brown in color and necrotic with scattered, minute nodules containing acid-fast bacteria. Many lymphatic glands showed slight enlargement. Other organs appeared from gross inspection to be normal.

Two guinea pigs were inoculated intraperitoneally and a rabbit intravenously with macerated nodules from the spleen of the above-mentioned guinea pig No. 587. The first guinea pig died 16 days and the second 30 days after inoculation, with lesions similar to those from which the inoculum was taken. The rabbit died 24 days after the injection and on post-mortem showed a miliary tuberculosis of the lungs, enlargement of the lymphatic glands, general thickening and nodular swelling of the omentum, and a few yellow nodules in the liver. Smears from the lungs and the nodular swellings in other organs exhibited moderately large numbers of acid-fast bacilli.

Many guinea pigs infected with 0.1- to 5- mg. doses of S forms isolated from the several BCG cultures have reacted in this manner. Some have died from the infection in as short a period as 12 days, some have survived for several months. Frequently more extensive involvement than that just described, has been encountered; other animals have died with gross lesions evident only in the omentum.

Characteristic S cultures were recovered from the lesions in many of these animals and progressive disease was produced on injection of the exudate or the tubercular tissue into other guinea pigs. In a few instances, notwithstanding the presence of large numbers of bacilli in the tissues and exudates

of animals dead of fatal progressive disease, it has been impossible to recover cultures or to produce infections with injected exudate. Such failures have been the exception.

It seems evident, therefore, that stable S forms recovered from BCG cultures are actively pathogenic. In the next section it is also shown that they possess a different antigenic structure from the R forms.

8. Antigenic Activity of BCG Cultures

In previous papers in this series it has been shown that S and R types of tubercle bacilli and other acid-fast species differ in antigenic structure as measured by complement-fixation (Rice (29), Rice and Reed (31), Reed, Rice and Gardiner (28), Rice, Orr and Reed (30).

The same technique as described in the former papers has been employed with BCG material. In complement-fixation, the total amount of complement reacting with the sensitized antigen and red cells to yield a final degree of haemolysis of 50%, has been used as the unit of comparison. The "specific fixation" with a particular antigen was obtained by subtracting from the amount of complement fixed by that antigen and an immune serum, the amount fixed by the same antigen in the presence of normal serum collected from the same animal previous to immunization. Although neither Shibata (34), Arena (1) or Boquet and Nègre (7) were able, in the presence of an excess of complement (two or more units), to demonstrate appreciable differences in the antigenic activity of extracts of BCG cultures and of preparations made from virulent strains, it was found possible by means of this more strictly quantitative technique to show that undissociated BCG cultures behave antigenically like other typical R cultures of tubercle bacilli, whereas dissociated S cultures, like other S cultures of tubercle bacilli, behave very differently from the R cultures.

Normal rabbits whose sera were low in nonspecific and anti-complementary properties, were given a series of inoculations with killed cultures of the various strains of BCG: two lots of animals with the characteristic BCG–R, Paris and Montreal strains; three lots of animals with the dissociated S forms of the Trudeau, Ottawa, and Alberta cultures; and one lot with a culture of the Alberta strain containing mostly R with a few S types.

These sera were tested in the presence of graded amounts of complement with a standard S antigen, an aqueous extract prepared from a typical S culture of tubercle bacilli and a standard R antigen, a similar extract made from a pure R culture of bovine tubercle bacilli. Both these antigens were used in previous work, Rice and Reed (31). The results of such a series of tests are assembled in Table IV. It is evident that the anti-R sera, the sera of rabbits immunized with the typical, or R, BCG of the Paris and Montreal strains, react to approximately the same degree with the S and the R antigens. Sera of three lots of rabbits immunized with dissociated S types from the Trudeau, Alberta, and Ottawa strains of BCG, the anti-S sera, react very

differently with the S and R antigens. With these anti-S sera, as shown in Table IV, the R antigen fixed from 45 to 60% of the amount fixed by the S antigen.

TABLE IV

A COMPARISON OF THE REACTIVITY OF TYPICAL S AND R MAMMALIAN TUBERCLE BACILLUS ANTIGENS WITH THE SERUM OF RABBITS IMMUNIZED WITH ORDINARY BCG–R ORGANISMS AND WITH DISSOCIATED S ORGANISMS AS MEASURED IN CUBIC CENTIMETRES OF COMPLEMENT FIXED SPECIFICALLY AT FIFTY PER CENT HAEMOLYSIS

Antiserum	Antigen	Specific fixation	S/R Index	Antiserum	Antigen	Specific fixation	S/R Index
		cc.				cc.	
BCG–R, Montreal (1)	S R	.0041 .0035	1.17	BCG–S, Trudeau	S R	.0064 .0030	2.13
BCG–R, Montreal (2)	S R	.0080 .0074	1.08	BCG–S, Alberta (1)	S R	.0032 .0014	2.28
BCG–R, Montreal (3)	S R	.0147 .0139	1.06	BCG–S, Alberta (2)	S R	.0143 .0087	1.64
BCG–R, Montreal (4)	S R	.0110 .0101	1.09	BCG–S, Alberta (3)	S R	.0143 .0066	2.17
BCG–R, Montreal (5)	S R	.0073 .0069	1.06	BCG–S, Ottawa (1)	S R	.0057 .0028	2.04
BCG–R, Paris	S R	.0090 .0080	1.13	BCG–S, Alberta (4)	S R	.0150 .0087	1.72
BCG–S and R Alberta (5)	S R	.0100 .0082	1.22				
Average	S R	.0091 .0083	1.10	Average	S R	.0098 .0052	1.88

In the earlier papers just quoted (29, 31), it was shown that in the sera of animals subjected to a long period of immunization with S tubercle bacilli, R antigens fix approximately half the antibodies fixed by the S antigen, whereas with the serum of animals similarly immunized with R organisms an equal amount of fixation occurs with R and S antigen. It was considered from the experimental data that the S organisms contain an S-specific and a species antigenic substance and that S antiserum contains a corresponding S antibody and a species antibody; while in contrast, the R organisms lack the S-specific antigen but possess the species antigen and the R antiserum contains only the species antibodies. Precisely the same arguments may be applied to the BCG data presented in Table IV. The cultures consisting of R types of BCG produced in rabbits only species antibodies which were fixed to an equal degree by known S and R tubercle bacillus antigens. The BCG cultures which had dissociated to S types, produced in rabbits both S-specific and species antibodies, as shown by the fact that the S antigen produced approximately twice as much fixation as the R antigen.

These data offer then, further confirmation of the R character of the original and typical strains of BCG. They also indicate that in the dissociation of the Trudeau, Alberta, and Ottawa strains, types have been produced which possess S-specific substances. Dissociation, as in other tubercle bacilli, has evidently been accompanied by a change in antigenic structure.

It is apparent from these experiments that the BCG as ordinarily used for immunization is, like other R types of tubercle bacilli, lacking in certain antigenic complexes present in virulent human or bovine cultures or in dissociated S forms of the BCG. To what extent the complement-fixing activity of tuberculous serum indicates resistance to the disease is unknown; the majority of workers do not consider such antibodies as essential elements in the defence against tuberculous infection. If any such connection does exist, however, the usefulness of an R vaccine such as BCG in protecting against virulent S organisms might be questioned, since no antibodies are developed against the S-specific substances. Even if complement fixing substances are not important in determining the degree of immunity, it is not improbable that the difference in antigenic structure may affect their general immune reactions.

Discussion

These results apparently indicate that BCG cultures consist of R forms of tubercle bacilli and, in agreement with earlier observations on other well established R cultures of tubercle bacilli, they have proved to be highly stable. Had observations not been continued for one to three years we would have been in agreement with the majority of students of these cultures that the R form is a fixed type, judging by all except one of the cultures of BCG under examination. Continued observation, however, indicates that under possibly exceptional circumstances, R to S dissociation may occur. It seems highly unlikely that this change will ever take place under the usual conditions of the cultivation of BCG for vaccine purposes. From this point of view it might be considered that the observation of dissociation gives no indication of danger in the use of BCG vaccine. It does however call attention to the necessity of the most rigid control in the preparation of the vaccine and to the possibility of dissociation taking place in the animal body. No new evidence or discussion of the latter point has been introduced in this paper.

Probably the indication that the ordinary BCG culture, the R form, is antigenically different from the virulent or S tubercle bacillus offers the more serious commentary on the BCG vaccine problem. The R variants of other bacterial species, the pneumococci, the colon-typhoid group, have been shown to produce little protective effect in inoculated animals (12). It would not be surprising therefore to encounter a similar condition in tubercle bacilli. More definitely it has been shown in this paper that the BCG, in common with other R forms of the species, is lacking in certain antigenic complexes, demonstrable by complement-fixation reactions, which are present in virulent or S forms and that the serum of animals treated with killed S organisms contains a specific S antibody. Animals treated with R organisms lack this specific antibody. It is impossible to draw conclusions concerning immunity from complement-fixing reactions but, as previously discussed, it seems reasonable to anticipate that the lack of a specific S antigenic substance in the R organisms may seriously affect this general immunizing reaction.

Conclusions

Five cultures of BCG have been shown to consist primarily or entirely of R type tubercle bacilli and to resemble R types from other sources.

One culture contained, when received, a few S types which were isolated. The other four cultures appeared to consist entirely of R types. After a long series of cultures particularly in slightly alkaline Proskauer and Beck's fluid, dissociation occurred and S types were isolated from two strains, no stable S types could be recovered from two additional strains.

The R or ordinary BCG produced only localized infections, the S forms produced fatal progressive tuberculosis in guinea pigs. The degree of tissue involvement in fatal cases was generally less than with typical virulent tubercle bacilli.

By means of complement fixation reactions it was shown that the S form, recovered from BCG cultures, like typical S virulent mammalian tubercle bacilli, contains a specific antigenic substance which is lacking in the ordinary BCG and other R forms of the species. Animals immunized with S organisms are shown to develop a specific S antibody which is not present in the serum of animals treated with R organisms or the ordinary form of BCG. It is suggested that this lack of antigenic substance in the BCG militates against its effectiveness as an immunizing agent.

Acknowledgments

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THE IODIMETRIC ESTIMATION OF ACETONE[1]

By W. H. Hatcher[2] and J. F. Horwood[3]

Abstract

The iodimetric estimation of acetone is directly related to two factors: (1) the enolization of acetone by alkali, and (2) the relative velocities of (1) and the reaction between alkali and iodine. The experiments necessary to emphasize these factors indicate the great rapidity of these two reactions at 25° C.

Introduction

The use of iodine and alkali in quantitative estimations has been the subject of much investigation. The present communication is a continuation of a previous study by one of us (3) and represents a co-ordinated system of experiments designed to explain certain apparent inconsistencies previously noted. These inconsistencies may be summarized as follows:—

(1) Sodium hydroxide, acetone and iodine in potassium iodide solution give iodoform, etc., the completeness of the reaction being dependent upon the excess of alkali, the order of addition of reagents being that given;

(2) In the order acetone, iodine, sodium hydroxide, completeness is abnormally delayed;

(3) In the order iodine, sodium hydroxide, acetone, completeness of estimation is never achieved.

To avoid a large bibliography, only those publications having a distinct bearing on the problem will be mentioned.

Procedure

Mixtures of acetone, sodium hydroxide and iodine were allowed to react in aqueous solution for a certain time, made just acid with dilute sulphuric acid, and immediately titrated with sodium thiosulphate. The conditions controlled were temperature, concentration, order of addition, and time of reaction.

Reagents

The *sodium hydroxide, iodine, iodate-free potassium iodide, and sodium* thiosulphate were of the highest grade of purity obtainable.

The acetone used was correct in boiling point and contained no trace of aldehyde or acid. A weighed quantity was dissolved in distilled water and aliquots measured therefrom. The burettes and pipettes used were carefully calibrated, blank experiments performed, and all precautions taken to ensure accuracy. The highest error in titration was four-tenths of one per cent; usually it was about one-quarter of that.

[1] *Original manuscript received November 27, 1933.*
Contribution from the Department of Chemistry, McGill University, Montreal, Canada.

[2] *Associate Professor, Department of Chemistry.*

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Results

For the sake of brevity the experiments are summarized in Table I; here are given the experiment numbers, the concentration of reagents, the temperature, and the total volume of solution. These concentrations are expressed in gram molecules per litre for acetone and sodium hydroxide, and in gram equivalents per litre for iodine. In each case the order of addition is acetone, sodium hydroxide, iodine.

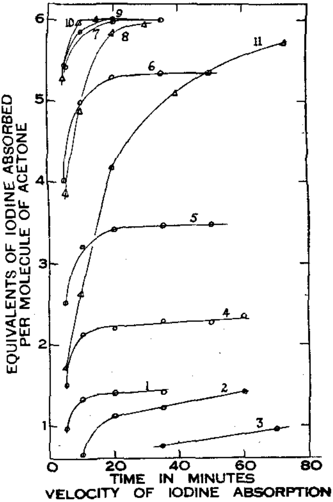
TABLE I

Expt. No.	Concentrations			Temp., ° C.	Total volume, cc.
	Acetone	NaOH	Iodine		
1	0.0024	0.024	0.0234	25	200
2	0.0008	0.008	0.0078	25	600
3	0.0004	0.004	0.0039	25	1200
4	0.0008	0.012	0.0078	25	600
5	0.0008	0.016	0.0078	25	600
6	0.0008	0.024	0.0078	25	600
7	0.0008	0.048	0.0078	25	600
8	0.0004	0.024	0.0078	25	600
9	0.0004	0.024	0.016	25	600
10	0.0008	0.048	0.016	25	600
11	0.0008	0.048	0.0078	0	600

The results at each time interval are not given but are found in Fig. 1, where the curves are numbered to correspond to the experiment numbers.

The experiments shown in Table I are correlated below together with the corresponding curves of Fig. 1, in order to show the influence of each factor:

I. Effect of dilution: Expts. 1, 2 and 3.
II. Effect of alkali concentration: Expts. 2, 4, 5, 6 and 7.
III. Effect of acetone concentration: Expts. 6 and 8.
IV. Effect of iodine concentration: Expts. 7 and 10, 8 and 9.
V. Effect of temperature: Expts. 7 and 11.



VELOCITY OF IODINE ABSORPTION

FIG. 1

In Fig. 1 the numbering of the curves corresponds to that of the experiments. Here time is plotted against gram equivalents of iodine used per gram molecule of acetone employed. Additional points left out of the curves are as follows: Curve 3, 1.42 equivalents in 315 min.; and curve 11, 5.90 equivalents in 130 min.

Mixtures having the concentrations of experiment No. 6 in Table I were made up in varying order of addition of reagents; the results are summarized in Table II.

TABLE II

—	Order of addition	Iodine absorbed, % of the theoretical	Time
(A)	Acetone, Alkali, Iodine	88	20 min.
(B)	Acetone, Iodine, Alkali	59.8	16 hr.
(C)	Alkali, Iodine, Acetone	54.6	41 hr.

NOTE: *Temperature, 25° C.*

Discussion of Results

A study of the curves in Fig. 1 reveals the following points: (i) a rapid reaction under favorable conditions; (ii) the apparent cessation of iodine absorption when the alkali concentration is less than 0.048 *N*. If the apparently final equivalents in Experiments 2, 4, 5, 6 and 7 be plotted against initial concentration of alkali, a straight line will be obtained which will indicate the final six equivalents of iodine when the alkali is about 0.03 *N*,—*i.e.*, about 35 mols of alkali for each one of acetone.

Table II shows the importance of the order of addition; this has further significance when the technique of addition is considered. In each case the first two reagents were added and the volume of solution was complete except for the addition of the third; the time of addition of all the reagents was about three minutes. In order (A), the acetone and alkali could stand together for as much as an hour without interfering with the final result; with order (B) the same is approximately true; whereas with order (C), delay in adding the acetone is fatal to the production of iodoform.

The reaction occurring here has been summarized in the well known equation:

$$4NaOH + 3I_2 + CH_3COCH_3 \rightarrow CHI_3 + 3NaI + CH_3COONa + 3H_2O$$

and is the basis of the Messinger method of acetone estimation (4). Since in neutral or acid solution acetone undergoes no rapid reaction such as occurs here, the effect of alkali on iodine solutions is obviously an important factor. An equally important factor is the action of alkali on acetone. Summarizing these we have the following reactions:

$$2K^+ + 2I^- + I_2 \rightleftarrows 2K^+ + I^- + I_3^- \qquad \text{rapid} \quad (1)$$

$$\left.\begin{array}{l} I_2 + OH^- \rightarrow HIO + I^- \\ \text{or} \\ I_2 + OH^- \rightarrow HIO^- + 2I^- \end{array}\right\} \quad (2)$$

or the HIO may exist as H^+ and IO^-. This, according to Skrabal, is very rapid (5).

The much slower reaction

$$3I_2 + 6OH^- \rightarrow IO_3^- + 5I^- + 3H_2O \quad (3)$$

enters subsequently.

According to Evans and Nicoll (1)

$$CH_3.CO.CH_3 \rightleftarrows CH_3.COH:CH_2 \quad (4)$$

and this iso-acetone may be increased to 26.5% by alkali concentration of 0.0369 *N*, further alkali not affecting this equilibrium.

A picture may therefore be presented explaining the orders of addition and the effects of alkali, as follows:

Order (A). Acetone becomes enolized in direct proportion to the alkali concentration, the minimum concentration of about 0.03 *N* found previously agreeing well enough with that of Evans and Nicoll. Inasmuch as with 0.048 *N* alkali the reaction was almost complete in 10 min. (Fig. 1, curve 10), this acetone-iso-acetone equilibrium must be rapidly adjustable. Concurrently with this enolization, the rapid formation of HIO (perhaps, rather than NaIO or IO^-) completes the reaction by iodination, oxidation and fission of the chain at the ethylenic bond.

Order (B). Here the two main reactions (numbers 2 and 4) are in conflict, the rapid formation of hypoiodite partially removing the hydroxyl ion so essential for enolization, since the NaOH concentration was initially 0.024 *N*.

Order (C). The considerations above apply here more fully, as reaction No. 2 is well under way before the acetone is added,—even within the three minutes of addition.

That the reaction between sodium hydroxide and iodine dissolved in potassium iodide is rapid has been shown by mixing the two rapidly and taking their conductivity immediately: well within three minutes the conductivity had dropped to a value which it later maintained for a considerable period (2).

Apart, therefore, from offering an explanation of the iodimetry of acetone, these experiments give an idea of the rapidity of (i) enolization of acetone by alkali and (ii) the reaction between alkali and iodine.

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CERTAIN REACTIONS OF GAMMA KETONIC ACIDS[1]

By C. F. H. Allen[2], J. B. Normington[3] and C. V. Wilson[4]

Abstract

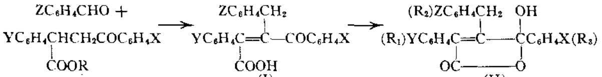
A considerable number of highly substituted acrylic acids or their lactols have been synthesized, and the Grignard reagent used to differentiate between the two possible structures. Acetyl chloride was found to be a satisfactory confirmatory agent, giving chlorides with the lactols, but not reacting with the open-chain acids. From the available evidence it is concluded that the differences may be attributed to *cis-trans* isomerism.

Two other series of ketonic acids were investigated with both reagents; the Grignard reagent indicated mostly open-chain structures. The use of acetyl chloride led to a variety of products; by varying the procedure, dimers of undetermined structure, unsaturated lactones, enolic acetates, and methyl esters were obtained.

Cyclohexanone gave cyclohexenyl acetate with acetyl chloride.

The mechanism of the reactions is discussed, as well as the evidence for the possible structures of derivatives of levulinic acid. A mechanism is suggested to account for the formation of enolic esters and unsaturated lactones of enolized ketonic acids.

When aromatic aldehydes are condensed by means of alkaline reagents with esters of α-aryl-β-aroyl-propionic acids, the resulting products have been found to be acrylic acids or their lactols (3, 4).



(I) (II)

It was noted (4) that when X = H, the acid only was produced, and that the latter showed no indications of a tautomeric cyclic structure. In order to learn whether there was any connection between the location and nature of substituent groups (X, Y, Z) and formation of open-chain or ring compounds, a considerable variety of compounds was prepared. The Grignard reagent was used to distinguish between the two types of structure. It was used in an apparatus designed to measure quantitatively both the amount of active hydrogen and addition (19, 20). In the earlier work it was found that the acids showed addition of one mole of reagent for each mole of gas evolved, whereas the cyclic substances added two moles per active hydrogen. A known unsaturated lactone of the same type showed two additions, indicating that the method was reliable with such substances. The results are recorded in Table I.

In two instances it was impossible to obtain check results. It will be noted that the mesityl homologue has the open-chain structure (one active hydrogen, one addition). Since 1,2-addition to mesitylenic ketones is un-

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Contribution from the Department of Chemistry, McGill University, Montreal, Canada. (This paper was read at the meeting of the Royal Society of Canada at Quebec, May, 1934.)

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TABLE 1

RESULTS OBTAINED WITH THE LACTOLS OR KETONIC ACIDS IN THE GRIGNARD MACHINE

R_1	R_2	R_3	Active H	Addition
Acrylic acid series				
C_6H_5	C_6H_5	C_6H_5	0.9	1.0
C_6H_5	C_6H_5	C_6H_4Cl-4	1.1	2.1
C_6H_5	C_6H_5	C_6H_4Br-4	1.0	1.9
Piperonyl	C_6H_5	C_6H_4Cl-4	1.1	2.1
C_6H_5	C_6H_4Cl-2	C_6H_5	1.1	1.1
C_6H_5	C_6H_5	$C_6H_4C_6H_5$-4	1.1	1.0
Anisyl	C_6H_5	C_6H_5	1.1	1.3
Anisyl	α-Furyl	C_6H_5	1.1	1.1
C_6H_5	Piperonyl	C_6H_4Cl-4	1.3	1.1
Anisyl	C_6H_4Cl-2	C_6H_5	1.6, 1.3, 1.4	1, 4, 1.8, 1.9
Anisyl	$C_6H_4OCH_3$-2	C_6H_5	*2.2, 1.9, 1.1, 1.6*	*0.3, 0.9, 1.6, 1.5*
C_6H_5	C_6H_5	Mesityl	1.1, 1.1	1.1, 1.0
C_6H_5	C_6H_5	$C_6H_4CH_3$-4	0.9	1.9
C_6H_5	C_6H_4Cl-2	C_6H_4Cl-4	1.1	2.0
Aryl aroyl propionic acids				
C_6H_5	C_6H_5		0.9	1.0
C_6H_5	C_6H_4F-4		1.1	1.1
C_6H_5	C_6H_4Cl-4		0.9, 0.9	1.6, 1.5
C_6H_5	C_6H_4Br-4		1.0	1.5
C_6H_5	$C_6H_4CH_3$-4		1.0	1.9
C_6H_5	Mesityl		1.0	1.1
Piperonyl	C_6H_4Cl-4		1.1	2.0
Benzal (7)	C_6H_5		1.1	1.2
α, γ-Diphenylcrotolactone (5)			0.1	2.0
α-Phenyl-β-benzyl-β-(4-chlorobenzoyl) propionic acid			1.1	1.0

known, in this instance, by analogy with the behavior of a similar unsaturated ketone (18), the reagent has presumably attached itself to the 1,4-positions in the conjugated system.

There are two possible explanations that may account for the types of products found, *i.e.*, ring-chain tautomerism* and *cis-trans* isomerism. At first sight the former might seem the more probable. The principal method hitherto successfully applied to determine the amount of cyclic form present in the ketonic acids, namely, titration with various strengths of barium hydroxide (37, 38, 39, 41, 42), could not be used with the compounds described in this paper, because of their insolubility. Also, if this were a case of mobile tautomerism it would have to be assumed that the Grignard reagent reacted more rapidly than any tautomeric change could take place. However, the results found fell very clearly into two classes, which would not be expected of such a variety of tautomeric substances. It would account for the varied values found in the two exceptions cited.

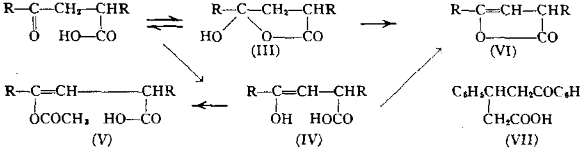
The alternative possibility is that of *cis-trans* isomerism, the acids having a *cis* structure giving rise to esters of both the open and the cyclic forms, which would obviously be impossible with a substance having a *trans* configuration. This would be in agreement with the observation that the open-chain acids never gave a single product derived from the cyclic form. The most conclusive proof would be the isolation of three esters, corresponding to the cyclic, *cis*, and *trans* forms, as accomplished by Lutz in the case of certain aroyl crotonic acids (24–28). It was shown in the first paper (3) that the lactols gave cyclic and open-chain esters, but in no case has it been possible to isolate the third. Geometrical isomers are often intraconvertible by means of exposure to sunlight under various conditions, and the *trans* modification is often colored; in this work substances of each class (I, II) were unchanged after six weeks' exposure and all were colorless. This may be inconclusive, since such properties are not necessarily universal.

It would seem unusual to find that a substituent group in one ring was a determining factor in giving rise to a *cis* or *trans* structure, especially since both types of products were found indiscriminately. However, Lutz has found that groups in rings do have a very marked effect in determining the configuration of diaroylethylenes, so not only is this objection without weight, but the parallel affords a strong argument that the differences are really of a stereochemical nature. Further, reduction of the 4-chloro homologue, known to be cyclic, gives a saturated acid which has an open-chain configuration, according to the Grignard results. Since a *cis* configuration forces the groups closer together (the reverse is true of open-chain saturated types, as Lutz found in analogous series), this evidence also favors a stereochemical explanation. It seems probable, therefore, that this is an additional example of *cis-trans* isomerism, the *cis* acids having a cyclic structure, which is impossible in the case of those having a *trans* configuration.

Since tertiary alcohols that have no hydrogen in a position available for dehydration form chlorides when treated with acetyl chloride, representatives of each class were treated with this reagent. It was found to form chlorides with those (II) that the Grignard reagent had shown to be cyclic (with one exception) but to be without action on those (I) having the open-chain form, thus acting as a confirmatory agent. The reaction with acetyl chloride is not a reliable criterion for distinguishing the enolic form in ordinary tautomerism, but these cases are not exactly comparable; moreover, if the substance has a cyclic structure, the product is a chloride, whereas an enol has never been known to form a chloride with this reagent.

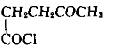
Two simpler saturated series were then investigated. The α-aryl-β-aroylpropionic acids were already available, since they had been made for use in the first part of the investigation, and the β-aroylpropionic acids were easily prepared. There was no evidence of a cyclic form of the latter, but in a few instances, the former gave, in the Grignard machine (Table I), reproducible intermediate values that could be interpreted as evidence for the existence of a mixture of the two possible forms (there is no opportunity for stereo- or geometrical isomerism). Two are apparently entirely cyclic.

In these two series, the hydroxyl group of any lactol (III) could be eliminated as water, so that dehydration would be anticipated with acetyl chloride and it would not be expected to be of value as a confirmatory agent. An enolic form (IV) is also a possibility that cannot be disregarded; it could not only yield an ester (V) but it could form an unsaturated lactone (VI) by loss of water with the carboxyl group.

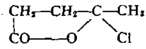


Varied results were obtained in both series. The prolonged action of acetyl chloride on the α-aryl-β-aroylpropionic acids brought about some kind of dimerization, the nature of which was not determined because of the intractability of the products. Neither the nature of the substituent groups nor the length of the chain seemed to have any effect, because the same types of products were obtained from the δ-ketonic acid (VII) as from the mesitoyl* γ-acid. Since addition reactions of the carbonyl group in a mesitoyl γ-acid are excluded, the dimerization, if it involves the group at all, must be through an enolic modification. There was one exception—the mesitoyl homologue gave an unsaturated lactone in addition to the substance of high melting point.

An unexpected result was obtained when the acetyl chloride solutions of these acids (including VII) were cautiously treated with methyl alcohol—methyl esters were formed. Easy esterification may be a property of ketonic acids, since Cowley and Schuette (10) have found that unless precautions were observed, alcoholic solutions of levulinic acid gave the 2,4-dinitrophenylhydrazone of the ester during the short time required to carry out the reaction. The production of esters may thus be accounted for, the hydrogen chloride formed from the acetyl chloride acting as a catalyst. An alternative possibility is that of interchange of radicals, even though the few recorded cases of interchange between an acid and an acid chloride took several hours (34, 36, 48), whereas the longest time involved in this series was 30 min. To explain the ease of formation of the esters, it would have to be assumed that (i) the acid chlorides are formed first, by a rapid interchange of radicals, and (ii) the acid chlorides have the open-chain structure (VIII). The only anomaly would then be Bredt's cyclic chloride (IX) of levulinic acid (9), which is discussed below.



(VIII)



(IX)

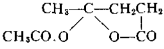
**Mesitoyl* = $-COC_6H_2(CH_3)_3$-2,4,6.

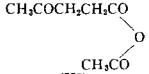
In the simplest series, the β-aroylpropionic acids, except the mesitoyl, gave unsaturated lactones; the mesitoyl gave an enolic acetate (V).

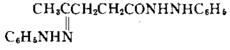
Since the products in the three series are not alike, the mechanism of the action of acetyl chloride must be different in the three. In the first series, in which it reacts only with those shown to be lactols by the Grignard reagent, the mechanism is similar to that of triphenylcarbinol, whatever that may be. In the other series it would be possible to account for the results by assuming that the acetyl chloride was first added to the carbonyl group (33); such an addition product could readily give rise to either enolic acetates or unsaturated lactones, both of which were found. Such an assumption is excluded because the mesityl compounds gave the same types of products, and no addition reactions of the carbonyl group of a mesitylenic ketone are known (13, 14, 20). This assumption was excluded long ago in the levulinic acid series by Bredt (9) who found that the esters did not react with acetyl chloride. Further, as previously mentioned, the halide did not react with the keto acids in the acrylic acid series described in this paper, which were shown by means of the Grignard machine to have an open-chain configuration. The remaining possibility is that of direct reaction with an enolic form; this seems plausible and accounts satisfactorily for the various types of products. The enol may form an ester directly or an unsaturated lactone by loss of water with the carboxyl group.

Lees (23) found that aliphatic ketones formed enol benzoates with benzoyl chloride but did not react with acetyl chloride. In this work it has been found that cyclohexanone reacts with acetyl chloride to form the known enol acetate (29). All efforts to prepare an enolic acetate or benzoate of acetomesitylene were unsuccessful, although it is known that this ketone is enolized by the Grignard reagent (20).

When levulinic acid is treated with acetyl chloride, it forms a chloride to which Bredt (9) finally assigned the cyclic structure (IX), because on treatment with silver acetate it gave "acetyl levulinic acid". The principal argument for a cyclic structure (X) for the latter was the production of the angelica lactones from it by loss of acetic acid, and the reverse—addition of acetic acid to the lactones to give the "acetate". The evidence by no means conclusively excludes an open-chain mixed anhydride structure (XI)—in fact, this structure seems the more probable one in view of the other known reactions and some of the facts presented above.


(X)

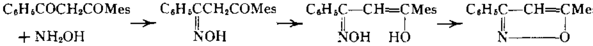

(XI)


(XII)

Bredt found that all the supposed cyclic derivatives of levulinic acid, on treatment with phenylhydrazine, gave the phenylhydrazone of levulinic phenylhydrazide (XII); this involves no unusual assumptions if the open-chain formulas are used. Further, silver levulinate and acetyl chloride gave

the same "acetate", a procedure which is employed to form mixed anhydrides. Wolff (50) found that "levulinic acid chloride" in carbon disulphide was easily brominated, and that when the reaction mixture was treated in the usual manner, β,δ-dibromolevulinic acid was produced. This is the ordinary bromination product of levulinic acid. The mechanism of formation of this bromination product is like that of any ketone having hydrogen in the position alpha to a carbonyl group.

It has long been known that lactones formed from enolized ketones are not regenerated when their solutions in alkalies are acidified, and that they are formed from the ketonic acids by the action of acetic anhydride, in exactly the same manner as ordinary ketones give enolic acetates; therefore, any evidence based on the lactones is inconclusive. As described above, the *mesitylenic ketones, which cannot form addition products, give both an* unsaturated lactone and an enol acetate, either of which could arise from an enolic form of the ketone, as outlined on page 385. In addition, benzoylmesitoylmethane readily gave an isoxazol; since mesityl ketones do not form oximes, the reaction undoubtedly proceeds as follows:



water being lost from an enolic modification.

In view of these facts, it seems extremely probable that lactone formation in the ketonic acids is brought about by the dehydration of an enolic form, and, therefore, in the case of levulinic acid the formation of the angelica lactones cannot be used as evidence for a cyclic form; whether the acetate is an intermediate product is uncertain. The available present evidence seems to be overwhelmingly in favor of open-chain structures for the levulinic acid series.

Experimental

A. The Chalcones and Related Compounds

All the required ketones were obtained by the usual methods. The yield of 2-chloro-5-methylacetophenone was increased from 34 to 85% by increasing the time of reaction to four hours (2). *p*-Carboxyacetophenone was obtained by heating *p*-bromoacetophenone with aqueous potassium cyanide in an autoclave, using a cuprous cyanide catalyst (12).

The unsaturated ketones* were prepared as previously described (3); the properties of the new compounds are given in Table II. The 4′-methyl homologue crystallized easily during the preparation if the temperature was kept below 30° C. (cf., Barat (6)). The 4′-carboxy derivative was made by shaking a cold mixture of a 10% solution of sodium hydroxide and 50 cc. of water; in a few minutes the sodium salt of the desired acid separated and was used in this form in the next step. The yield was 97%. A sample was converted to the free acid, m.p. 232° C.** (43).

* *The benzalacetomesitylene was prepared by Mr. E. T. Henry, and the p-fluoro homologue by Mr. G. K. Dodd; their assistance is gratefully acknowledged.*

** *Melting points are corrected.*

TABLE II

PROPERTIES OF THE CHALCONES AND THE DIBROMIDES

RCH=CHCOR′			Dibromides			
R	R′	B.p., ° C.	M.p., ° C.	Crystal form	Calcd., %	Found, %
C_6H_5	2-$ClC_6H_3CH_3$-5	195–200/6 mm.	117	Needles	Cl, 8.8	8.6
					Br, 39.8	39.5
C_6H_5	2-$CH_3C_6H_3(i\text{-}C_3H_7)$-5	205–210/12 mm.	140–1	Needles	Br, 37.7	37.5
		M.p., ° C.				
Piperonyl	C_6H_4Cl-4	Needles, 128	—	—	Cl, 12.4	12.2
			152 dec.	Plates	Cl, 8.0	7.9
					Br, 35.8	35.5
C_6H_5	Mesityl	—	131	Rods	Br, 39.0	38.8
C_6H_5	C_6H_4F-4	Plates, 76–77	—	—	C, 79.8	79.8
					H, 4.8	4.7

3,4-Methylenedioxybenzoyl-*p*-chlorobenzoylmethane (*A*) and benzoylmesitoylmethane (*B*) were prepared from the corresponding chalcone dibromides by the standard procedure* (1). α-Bromobenzal-2,4,6-trimethylacetophenone (*E*) was formed when the corresponding dibromide was treated with hydroxylamine by the procedure that has been shown to give isoxazols of known configuration (49), whereas 3-*p*-chlorobenzoyl-5-piperonylisoxazol (*C*) was obtained from the corresponding dibromide under the same conditions. The latter also resulted by the action of hydroxylamine on the diketone; 3-mesityl-5-phenylisoxazol (*D*) was obtained in a similar manner.

TABLE III

PROPERTIES OF THE DIKETONES, ISOXAZOLS, AND MONOBROMIDE

Substance	Formula	M.p., ° C.	Crystal form	Calcd., %		Found, %	
				C	H	C	H
A†	$C_{16}H_{11}O_4Cl$	151	Leaflets	63.5	3.6	63.4	4.0
B‡	$C_{18}H_{18}O_2$	84	Plates	81.2	6.7	81.1	7.0
C	$C_{16}H_{10}O_3NCl$	180	Leaflets	64.0	3.3	63.8	3.8
D	$C_{18}H_{17}ON$	76	Plates	82.1	6.4	82.3	6.8
E	$C_{18}H_{17}OBr$	73	Plates	65.4	5.5	65.0	5.6

NOTE: † *Copper derivative, m.p. 290–292° C., dec.* ‡ *Copper derivative, m.p. 185° C., dec.*

B. The Nitriles, Acids and Esters of the α-Aryl-β-aroyl propionic Acid Series

These were also obtained by previously described methods; their properties are summarized in Tables IV, V and VI.

* *The authors are indebted to Mr. A. J. Cameron for the preparation of the substances in the piperonyl series, and to Mr. W. R. Carmichael for the mesityl compounds. Their co-operation is gratefully acknowledged.*

The preparation of the nitrile from the 4′-phenyl homologue required four hours' heating at the boiling point, using 20 cc. of alcohol and 5 cc. of chloroform per gram of unsaturated ketone; the 4′-carboxy derivative was obtained by using a mixture of the substances in the ratio of 3 moles of potassium cyanide and 1.5 moles of acetic acid to 1 mole of the sodium salt of the ketone, and stirring for two hours at 75° C. The clear solution was then acidified with acetic acid.

TABLE IV

PROPERTIES OF THE NITRILES, $RCH(CN)CH_2COR'$

R	R′	M.p., ° C.	Crystal form	Yield, %	Formula	Calcd., %	Found, %
C_6H_5	C_6H_4F-4	102	Needles	90	$C_{16}H_{12}ONF$	C 75.9 H 4.7	75.6 4.6
C_6H_5	$C_6H_4C_6H_5$-4	176	Prisms	80	$C_{22}H_{17}ON$	N 4.8	4.5
C_6H_5	$C_6H_4CH_3$-4	80	Needles	85	$C_{17}H_{15}ON$	N 5.6	5.7
C_6H_5	$C_6H_4NO_2$-4	155	Rods	75	$C_{16}H_{12}O_3N_2$	N 10.0	9.5, 9.7
C_6H_5	C_6H_4COOH-4	239	Plates	90	$C_{17}H_{13}O_3N$	N 5.0	5.2
C_6H_5	2-$ClC_6H_3CH_3$-5	76-77	Needles	85	$C_{17}H_{14}ONCl$	N 4.9	5.1
C_6H_5	Mesityl	77-78	Needles	80	$C_{19}H_{19}ON$	N 5.2	5.4
Piperonyl	C_6H_4Cl-4	129	Leaflets	80	$C_{17}H_{12}O_3NCl$	Cl 11.3	11.1

TABLE V

PROPERTIES OF THE ACIDS, $RCH(COOH)CH_2COR'$

R	R′	M.p., ° C.	Crystal form	Yield, %	Formula	Calcd., %		Found, %	
						C	H	C	H
C_6H_5	C_6H_4F-4	161	Plates	80	$C_{16}H_{13}O_3F$	70.5	4.7	70.3	4.5
C_6H_5	C_6H_4Br-4	160	Plates	84	$C_{16}H_{13}O_3Br$	57.6	3.9	57.8	4.0
C_6H_5	$C_6H_4CH_3$-4	152	Leaflets	90	$C_{17}H_{16}O_3$	75.4	6.2	75.7	6.1
C_6H_5	Mesityl	172	Cubes	50	$C_{19}H_{20}O_3$	77.0	6.7	76.8	6.6
Piperonyl	C_6H_4Cl-4	190	Leaflets	80	$C_{17}H_{13}O_5Cl$	61.3	4.2	61.2	4.2

TABLE VI

PROPERTIES OF THE ESTERS, $RCH(COOCH_3)CH_2COR'$

R	R′	M.p., ° C.	Crystal form	Yield, %	Formula	Calcd., %		Found, %	
						C	H	C	H
C_6H_5*	$C_6H_4CH_3$-4	112	Plates	88	$C_{18}H_{18}O_3$	76.6	6.4	77.0	5.9
C_6H_5*	$C_6H_4C_6H_5$-4	157	Needles	70	$C_{23}H_{20}O_3$	80.2	5.8	79.7	5.8
C_6H_5†	$C_6H_2(CH_3)_3$-2,4,6	60-61	Rods	95	$C_{21}H_{24}O_3$	77.4	7.1	77.3	7.1
C_6H_5†	C_6H_4F-4	101	Rods	80	$C_{17}H_{15}O_3F$	71.3	5.2	71.7	4.8
C_6H_5*	$C_6H_4NO_2$-4	104	Cubes	Very low	$C_{17}H_{15}O_5N$	N	4.4	N	4.0
C_6H_5*	$C_6H_4COOCH_3$-4	110	Plates	80	$C_{19}H_{18}O_5$	69.9	5.5	70.3	5.7
C_6H_5†	2-$ClC_6H_3CH_3$-5	80	Rods	94	$C_{18}H_{17}O_3Cl$	68.3	5.5	68.4	5.7
Piperonyl*	C_6H_5	121	Rods	66	$C_{18}H_{16}O_5$	69.2	5.1	68.8	5.5
Piperonyl*	C_6H_4Cl-4	109	Rods	72	$C_{18}H_{15}O_5Cl$	Cl	9.9	Cl	10.0

** Directly from nitrile; † through the acid.*

C. Aroylpropionic Acids and Esters

These were all prepared by the Friedel-Crafts reaction from succinic anhydride and the appropriate hydrocarbon (46). The three naphthalene derivatives were supplied through the kindness of Dr. L. F. Fieser, whose courtesy is gratefully acknowledged.

TABLE VII

PROPERTIES OF THE β-AROYLPROPIONIC ACIDS AND METHYL ESTER, $RCOCH_2CH_2COOH$

Aryl group	M.p., ° C.	Crystal form	Formula	Calcd., %		Found, %	
				C	H	C	H
C_6H_4Cl-4	131	Plates	$C_{10}H_9O_3Cl$	56.6	4.2	56.3	4.3
Mesityl	107	Plates	$C_{13}H_{16}O_3 . 1/2H_2O$	70.9	7.7	70.8	7.4
(Ester)C_6H_4Cl-4	63	Leaflets	$C_{11}H_{11}O_3Cl$	58.4	4.5	58.8	4.5

D. The Acrylic Acid Series

The new lactols or ketonic acids, except the carboxy-substituted ones, were obtained essentially as previously described (3, 4). To obtain the ester, the components were refluxed for 10 min. only, using 2-gm. lots; the sodium salt which separated, was removed, and acidified with acetic acid. When it was dissolved in sodium hydroxide, or in the large amounts used in the preparation, the acid was obtained on acidification. It was not found possible to condense salicylic aldehyde with any of the γ-keto esters.

In general, the acids were insoluble in sodium bicarbonate. They all dissolved in dilute sodium hydroxide, but formed oils with a 10% solution; cumarin was found to act likewise. They gave brilliant colors with concentrated sulphuric acid—this was especially marked with those containing ether groups.

TABLE VIII

PROPERTIES OF THE LACTOLS OF KETONIC ACIDS*

R_1	R_2	R_3	Crystal form	M.p., ° C.	Formula	Calcd., %		Found, %	
						C	H	C	H
C_6H_5	C_6H_5	Mesityl	Plates	250, d.	$C_{26}H_{24}O_3$	81.2	6.2	81.1	6.4
Piperonyl	C_6H_5	C_6H_4Cl-4	Needles	153	$C_{24}H_{15}O_5Cl$	68.8	3.7	69.0	3.8
Piperonyl	C_6H_5	C_6H_5	Rods	138	$C_{24}H_{18}O_5$	74.6	4.7	74.4	5.0
C_6H_5	C_6H_5	$C_6H_4C_6H_5$-4	Needles	144	$C_{29}H_{22}O_3$	83.2	5.3	82.9	5.3
C_6H_5	C_6H_5	$C_6H_4CH_3$-4	Prisms	133	$C_{24}H_{20}O_3$	80.9	5.6	80.6	5.8
C_6H_5	C_6H_5	C_6H_4COOH-4	Needles	240	$C_{24}H_{18}O_5$	74.6	4.7	74.8	4.4
C_6H_5	C_6H_5	$C_6H_4COOCH_3$-4	Plates	137	$C_{25}H_{20}O_5$	75.0	5.0	75.3	5.2
C_6H_5	C_6H_4Cl-2	C_6H_4Cl-4	Needles	147	$C_{23}H_{16}O_3Cl_2$	Cl	17.3	Cl	16.9
Anisyl	$C_6H_4OCH_3$-2	C_6H_5	Needles	126	$C_{25}H_{22}O_5$	—	—	—	—
C_6H_5	C_6H_4Cl-2	C_6H_5	Rods	98	$C_{23}H_{17}O_3Cl$	Cl	9.4	Cl	9.5
Anisyl	C_6H_4Cl-2	C_6H_5	Needles	154	$C_{24}H_{19}O_4Cl$	Cl	8.7	Cl	8.7
Anisyl	α-Furyl	C_6H_5	Needles	121	$C_{22}H_{18}O_5$	72.9	5.0	72.4	4.9

*
$$\begin{array}{l} \quad\; R_2CH_2 \\ \qquad\;\, | \\ R_1C{=}C{-}COR_3 \\ \quad\;\, | \\ \quad\; COOH \end{array}$$

As stated in the introduction, the method of indirect titration was of no value. The substances were insoluble in any excess of $N/100$ barium hydroxide, hot or cold; if enough alcohol was added to yield a clear solution, all gave values corresponding to 100% free acid. In the hope of obtaining results of value, all the substances were treated under the same conditions; the sample was dissolved in 3 cc. of methyl alcohol, 25 cc. of base added, and the excess found by back-titration. In most cases, the addition of the alkali solution caused the formation of a precipitate, so the values obtained are probably more a measure of solubility relation than anything else. The figures ranged from 45 to 95% of acid, open-chain form; the actual results were considered inconclusive, and so are omitted here. Under the same conditions, benzoic acid showed 100%, and cumarin 40%, acid.

E. Action of the Grignard Reagent

The results obtained are summarized in Table I. The values are considered accurate to 10%, except where ether groups are present.

In the case of all the β-aroylpropionic acids there was one active hydrogen and one addition, so the acids are not listed separately. The aroyl groups were benzoyl, *p*-methyl- and *p*-chlorobenzoyl, mesitoyl, 2-acenaphthoyl, 6-methylnaphthoyl-2, and 2,6-dimethylnaphthoyl-1. All formed sparingly soluble magnesium complexes, which made it necessary to prolong the treatment for several hours. An apparent anomaly was encountered with the mesitoyl compound; the active hydrogen was 1.5, 1.6, 1.6, with 0.9 addition in each case. This agrees with the values found on combustion (Table VII), where the acid is indicated as containing water of crystallization.

F. Action of Acetyl Chloride

a. Chlorides in the acrylic acid series. In addition to the tertiary chlorides described in the first paper (3), a homologue was obtained from the *p*-carboxy lactol; m.p., 197° C. Calcd. for $C_{24}H_{17}O_4Cl$: Cl, 8.7%. Found: Cl, 8.7%.

The acids that were indicated as having an open-chain configuration by the Grignard machine were unchanged by acetyl chloride.* The acid chloride of the 4′-phenyl homologue was obtained by the use of phosphorus pentachloride. On refluxing a benzene solution of 1 gm. of the latter with 2 gm. of the keto acid for an hour, removing the liquids *in vacuo* while heating on the steam bath, and recrystallizing from ether-petroleum ether, the chloride was obtained, m.p. 150° C. Calcd. for $C_{29}H_{21}O_2Cl$; Cl, 8.1%. Found: Cl, 7.9%. It formed a *p*-bromoanilide (4), m.p. 182° C.

Since tertiary chlorides like trityl chloride form substituted anilides with aromatic amines (11, 16), the formation of a *p*-bromoanilide is of no use for distinguishing the two series. Only one cyclic chloride, the one from the 4′-chloro homologue, was used, and it easily gave a substituted *p*-bromoaniline, m.p. 176° C. Calcd. for $C_{29}H_{21}O_2NClBr$: Hlg., 21.7%. Found: Hlg., 22.1%.

* *In this connection it may be noted that β-benzoylpropionic acid can be recrystallized unchanged from thionyl chloride and phosphorus trichloride.*

b. The α-aryl-β-aroylpropionic acids. When acetyl chloride solutions of these acids were allowed to evaporate spontaneously, fine crystalline powders were left. They were insoluble in the common solvents except dioxane, and insoluble in sodium hydroxide. The product from α-phenyl-β-(*p*-chlorobenzoyl) propionic acid, m.p. 235° C. with decomposition, showed two active hydrogens and one addition in the Grignard machine. Calcd. for $C_{32}H_{24}O_5Cl_2$: C, 68.7; H, 4.3; Cl, 12.7%; mol. wt., 559. Found: C, 68.8, 69.0; H, 4.7, 4.3; Cl, 12.8%; mol. wt., 525. The other homologues have the following melting points (all with decomposition): phenyl, 295° C.; *p*-fluorophenyl, 247° C.; *p*-methylphenyl, 270° C.

When the procedure was altered by destroying the excess of solvent by cautious addition of methyl alcohol, after a small amount of the high melting solid had separated, the solution deposited the methyl ester of the acid on evaporation. Thus, the ester from the simplest member, α-phenyl-β-benzoylpropionic acid, melted at 102° C.; it was at first thought to be the known α,γ-diphenyl-crotolactone, m.p. 103° C. (5), but a mixed melting point was 85–90° C. It did not decolorize bromine and proved to be identical with an authentic specimen of the ester.

There was but one exception in the series—the mesityl homologue gave a crotolactone; it formed needles, m.p. 126° C., decolorized bromine instantly, and dissolved in sodium hydroxide solution; on acidification α-phenyl-β-mesitoylpropionic acid was obtained. The lactone is soluble in the common solvents. Calcd. for $C_{19}H_{17}O_2$: C, 82.3; H, 6.1%. Found: C, 82.0; H, 6.5%.

β-Phenyl-γ-benzoylbutyric acid. This δ-ketonic acid also gave an ester when treated as indicated above. A trace of a solid of high melting point was formed by the action of acetyl chloride alone, but most of the acid was recovered unchanged. Barat (6) stated that he obtained a lactone by such a procedure, but he gave no experimental details, and his statement was not confirmed.

α-Phenyl-β-benzyl-β-(4-chlorobenzoyl) propionic acid was formed by the reduction of the corresponding acrylic acid with zinc dust and 80% acetic acid. It formed rods, m.p. 173–174° C., soluble in ether, alcohol, and benzene. Calcd. for $C_{23}H_{19}O_3Cl$: C, 73.0; H, 5.0%. Found: C, 72.6; H, 4.7%.

c. The β-aroylpropionic acids. (I) γ-(*p*-Chlorophenyl) crotolactone. A solution of 5 gm. of β-(*p*-chlorobenzoyl) propionic acid in 10 cc. of acetyl chloride was refluxed for two hours; on cooling, yellow needles separated. The color could not be completely removed by repeated recrystallization or by use of decolorizing carbon. It is soluble in the usual organic solvents, but insoluble in sodium carbonate solution. A bromide-bromate titration indicated 1.06 ethylenic linkages. Calcd. for $C_{10}H_8O_2Cl$: C, 61.5; H, 4.1%. Found: C, 61.2; H, 4.0%.

(II). Enol acetate of β-mesitoylpropionic acid. When β-mesitoylpropionic acid was treated in a similar manner, the solution became dark red, and purplish-red needles were deposited, m.p. 288–290° C. This substance may

be one of the "Pechmann dyes", some of which are known to be formed in an analogous manner (22). Calcd. for $C_{26}H_{24}O_4$: C, 78.0; H, 6.0%. Found: C, 78.1; H, 6.2%. The enol-acetate was obtained from the filtrate, and had a pink tinge which was never removed. It dissolved easily in sodium carbonate solution and was re-precipitated by acids. A bromide-bromate titration indicated 1.1 double bond. Calcd. for $C_{15}H_{18}O_4$: C, 68.6; H, 6.8; $C_2H_3O_2$, 22.5%. Found: C, 68.2; H, 6.6; $C_2H_3O_2$, 22.3%.

d. Cyclohexanone, cyclohexenyl acetate. A solution of 10 cc. of acetyl chloride and 10 gm. of cyclohexanone was refluxed for two hours and fractionally distilled; 5 gm. of a liquid, b.p. 175–178° C. at 763 mm. Hg. was collected. Since only the boiling point is given in the literature, a sample was prepared as directed by Mannich (29), and all properties compared; cyclohexyl acetate is also included in the table, for comparison.

TABLE IX

PROPERTIES OF CYCLOHEXANE DERIVATIVES

Substance	B.p., ° C. 763 mm.	D^{20}_{20}	n^{20}_D	M_D	
				Calcd.	Found
Cyclohexenyl acetate					
By above method	178	0.9726	1.4612	39.11	39.22
By Mannich's	178	0.9725	1.4610	39.11	39.20
Cylcohexyl acetate	174	0.9782	1.4440	38.59	38.56

G. Acetomesitylene

In an apparatus from which moisture was excluded, a mixture of 20 gm. each of acetomesitylene and *p*-chlorobenzoyl chloride was refluxed for 10 hr.; hydrogen chloride was slowly evolved. After washing with dilute sodium carbonate solution, which removed 1 gm. of *p*-chlorobenzoic acid, and fractionating the oil *in vacuo*, 11 gm. of ketone was recovered, and 7 gm. of *p*-chlorobenzoic anhydride was obtained. The residue was a pitch and there was no evidence of an ester. The small amount of water necessary for the reaction may have been fortuitously present or derived from the decomposition giving rise to the residue. From similar runs with benzoyl chloride, a complete separation of the mixture of ketone and benzoic anhydride that distilled together was almost impossible. However, there was no evidence of ester.

Acknowledgment

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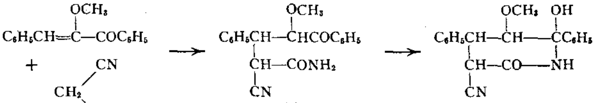
THE ADDITION OF CYANOACETAMIDE TO α-METHOXY-BENZALACETOPHENONE[1]

BY C. F. H. ALLEN[2] AND J. A. SCARROW[3]

Abstract

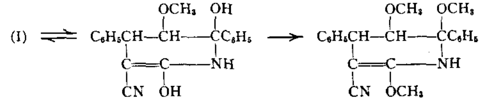
When attempts were made to add substances of the type HA to α-methoxybenzalacetophenone in the presence of an alkaline catalyst, it was found that the components were largely recovered unchanged, except in the case of cyanoacetamide; the addition product from the latter is a hydropyridine. The hydropyridine gives two distinct series of reaction products; in the first, the substances are all pyridine derivatives and always contain a methoxyl group. In the second, partial degradation has taken place, giving rise to cyclic derivatives of a δ-ketonic acid.

When cyanoacetamide was added to benzal α-methoxyacetophenone in the presence of a trace of sodium methylate, a hydropyridine (I) was obtained, instead of the isomeric open-chain δ-ketonic nitrile (II).



(II) (I)

This addition product had no free carbonyl group, since it did not form an oxime, a semicarbazone, or a phenylhydrazone; it was soluble in sodium hydroxide and on methylation of the sodium salt with methyl sulphate gave the trimethylether (IV).



(III) (IV)

The formation of the ether would point to the tautomeric structure (III) for the addition product, but other reactions are interpreted more satisfactorily by the ketonic formula. In the following description of the substance and its reactions it will be noted that the methoxyl group was never eliminated (except on treatment with sulphuric acid, which led to an entirely different series of products) and consequently it was impossible to convert the pyridine derivative into known substances. The assumption that they are pyridine derivatives is based on their consistent reactions and properties, and by analogy with a homologous series (XI) with a hydrogen in place of

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Contribution from the Department of Chemistry, McGill University, Montreal, Canada. (This paper was read at the meeting of the Royal Society of Canada at Quebec, May, 1934.)
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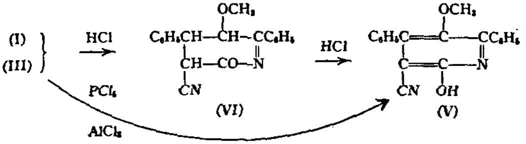
[3] *Graduate Student and Demonstrator in Chemistry, McGill University.*

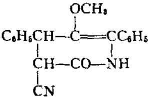
the methoxyl group (18). In all instances the reactions were strictly comparable; if unknown, they were determined in the course of this investigation.

The most characteristic reaction product was the highly substituted pyridine derivative (V); this substance was formed when phosphorus pentachloride or aluminium chloride was allowed to react with (I), but it appeared in nearly all reactions in varying amounts. Since the final ? step is a dehydrogenation, it is probable that the oxygen of the air removed the two

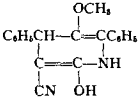


(VI) (V)

atoms of hydrogen during the prolonged time required for the usually slow reactions. For example, hydrogen chloride in chloroform first gave the pyridine (VI) by dehydration, but on standing several weeks (V) was obtained. Several alternative structures are possible for these substances; thus (VI) may be written as (VII) or (VIII). Since partially reduced pyridines are undoubtedly tautomeric substances, the formulas ascribed are a matter of individual preference.

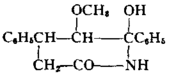


(VII)

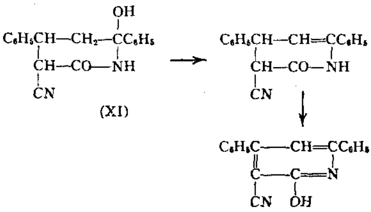


(VIII)

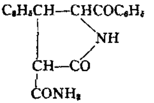
In addition to the hydroxypyridine (V), small amounts of (IX) and a lactam (X) were isolated from the mixture resulting from the action of anhydrous aluminium chloride. The homologous substance (XI), on similar treatment, gave the corresponding pyridine and tetrahydropyridine four times as rapidly.



(IX)

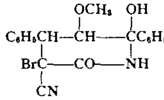


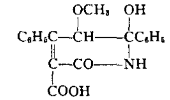
(XI)

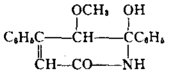


(X)

The action of bromine gave a complex mixture, from which were separated a bromo substitution product (XII), ammonium bromide, and three bromine-free products; two of the latter were readily identified as hydroxypyridine (V) and starting material (I). When the sodium salt of (I) was brominated, it gave a considerable quantity of (XII) and two bromine-free products; one of these was an acid (XIII), which lost carbon dioxide on warming to form the other (XIV), which was the third of the bromine-free products mentioned above. (XIII) is readily accounted for by hydrolysis and dehydrogenation; the amounts isolated were too small for further study.

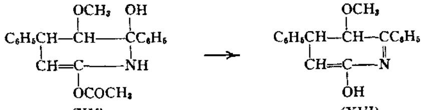

(XII)


(XIII)


(XIV)

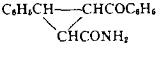
The bromine in (XII) was very unreactive; the substance dissolved easily in alcoholic alkali and was precipitated by acid practically unchanged, even after boiling with a $N/2$ solution of alcoholic potash for 2.5 hr. In boiling acetyl chloride it was slowly converted into the hydroxypyridine (V).

When attempts were made to dehydrate the addition product (I) with acetic anhydride in the presence of either sulphuric acid or pyridine, an acetate (XV) resulted, during the formation of which the nitrile group had disappeared. The acetyl residue was easily hydrolyzed with alcoholic sulphuric acid, but the process was accompanied by dehydration to yield (XVI). The structures assigned are subject to revision but seem to fit the available evidence better than any of the other possibilities. Kohler and Souther (18, pp. 2907-2908) described essentially similar results with (XI), and were able to detect the cyanide ion in one instance.

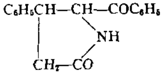

(XV) (XVI)

When the addition product was warmed in moderately concentrated sulphuric acid, a crystalline product and a gum were obtained on dilution. After exhausting all anticipated possibilities it was found that the solid formed a dinitrophenylhydrazone; therefore a free carbonyl group must have been present, and the six-membered ring structure of (I) must have been destroyed. As the cyano and methoxyl groups had also disappeared, the most probable structures were considered to be those of a cyclopropane

(XVII) or a lactam (XVIII). A stereoisomer, which gave the same dinitrophenylhydrazone resulted on treating an alcoholic solution of the substance with hydrogen chloride.



(XVII)



(XVIII)

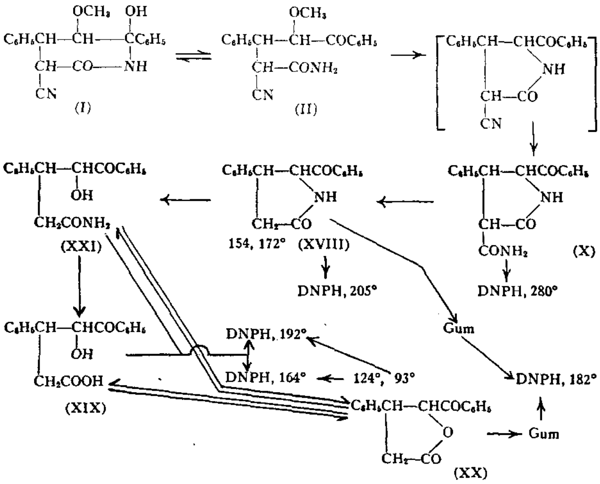
The substance was unaffected after boiling with phosphorus pentoxide in xylene, which made the cyclopropane-amide formula seem less probable. The solid dissolved very quickly when warmed with alkalies, ammonia being evolved, but the process was accompanied by complete decomposition. Since the nitrogen-free hydrolysis product of (XVIII) would be the hydroxy acid (XIX), which Kohler had already shown (14) to be very sensitive to both acids and bases, attention was turned to the gum; it gave a mixture of three dinitrophenylhydrazones. Two of these were isomeric and found to be identical with those prepared from the pair of stereoisomeric lactones (XX) or hydroxyacid (XIX), showing that the gum contained substances having the skeleton of a δ-ketonic acid. Some of the original gum was next carefully extracted with dilute alkali, and on acidification, the hydroxyacid and both lactones were separated.

When the lactones were treated with sulphuric acid in a similar manner, a gum resulted, from which a dinitrophenylhydrazone identical with the third one above was prepared. The analytical results indicated that it must be derived from some sort of a secondary product. On similar treatment, the lactam (XVIII) yielded a gum, that gave the same dinitrophenylhydrazone.

Attempts to synthesize the lactam by the action of ammonia on the lactone were unsuccessful. At ordinary temperatures a substance with almost the same melting point resulted, but the analyses indicated an addition product, possibly a hydroxyamide (XXI), which regenerated the lactone when treated with acetic anhydride. When treated with dinitrophenylhydrazone it gave the same dinitrophenylhydrazones as the lactones.

The course of the reaction from the addition product to the lactones may proceed in several ways. Since hydropyridines are always in equilibrium with open-chain compounds, the keto-amide (II) is undoubtedly the starting point. Whether hydrolysis of the cyano group or ring closure by loss of alcohol to form a cyano-lactam occurs first is uncertain. The latter possibility must be considered since a substance with properties corresponding to the amido-lactam (X) was isolated from the action of aluminium chloride

on (I). The series would then be (I) ⟶ (II) ⟶ cyano-lactam ⟶ (X) ⟶ carboxylactam ⟶ lactam (XVIII) ⟶ hydroxyamide (XXI) ⟶ lactone (XX) or hydroxyacid (XIX)



Cyanoacetamide was the only substance that it was found possible to add to α-methoxybenzalacetophenone by any procedure. The list of compounds used comprised benzyl and *p*-nitrobenzyl cyanides, desoxybenzoin, malonic, cyanoacetic, phenylacetic, and acetoacetic esters, nitromalonic aldehyde, anthrone, and cyclohexanone. A survey of the literature on this type of addition reactions indicates that cyanoacetamide adds most easily, judged by the time required and yield of product. *p*-Nitrobenzyl cyanide which has also been reported (1) to add readily, was recovered unchanged. Two suggestions for this lack of reaction may be considered. The first is the *presence of a substituent in the alpha position*, which is known to retard addition reactions to conjugated systems. Since it is only a slowing down of the rate it does not stop the reaction in the cases known, in which alpha substituents have been bromine (13, and experimental part of this paper), methyl (16), phenyl (9, 10, 11, 12), and carboalkoxy (5, 21), and the addends esters, ketones, nitriles, and aliphatic nitro compounds. The second is the

strongly positive nature of the methoxyl group. No quantitative data are available but Barat has stated that the more negative the ketonic end of the conjugated system, the more readily addition took place (3). In the above list of substituents methyl is the only (weakly, if at all) positive group, which would make the second suggestion seem more plausible. On the other hand, the real reason why the reaction takes place with cyanoacetamide only is probably because of the formation of the sparingly soluble hydropyridine. As this slowly separates it disturbs the equilibrium. Small amounts accumulate and the reaction proceeds in the direction of the cyclic compound. This explanation would also account for the ease with which Barat obtained hydroxypyridines in analogous instances.

Experimental

A. *The Methoxy Ketones*

*ω-Methoxyacetophenone**, $CH_3OCH_2COC_6H_5$. The published directions are incomplete, in that sufficient details of the procedure, which has to be followed very carefully, are not given. To a solution of phenyl magnesium bromide (from 33 gm. of bromobenzene, 4.5 gm. of magnesium and 150 cc. of ether) was gradually added, with cooling, 10.4 gm. of methoxyacetonitrile† (23) in 10 cc. of ether). A brittle mass of the magnesium salt was formed, and this was pulverized with a heavy glass rod. After standing overnight, the mixture was added to 200 gm. of cracked ice and 20 cc. of concentrated sulphuric acid, the oily layer removed and the aqueous solution extracted with ether. The solvent was removed from the combined solutions, leaving a 70% yield of crude ketone, which was fractionated twice *in vacuo*. A fraction boiling at 100–130° C. at 15 mm. was obtained; yield, 40%. This fraction was then extracted with hot water (the two layers separated easily), which on chilling deposited large yellow rods. These could be used to inoculate an alcoholic solution of the oil obtained from the distillation. The purified ω-methoxyacetophenone had a melting point of 7.0–7.5° C.; b.p., 118–120° C. at 15 mm., 228–230° C. at 760 mm. The solubility in hot water is about 2.6 gm. per 100 cc. The semicarbazone, m.p. 129° C., and the 2,4-dinitrophenylhydrazone were prepared in the usual manner. The latter formed orange stubby rods, m.p. 191–192° C.; the analysis has been given elsewhere (24).

α-Methoxybenzalacetophenone. Though now relatively easily prepared (15) all this ketone used was obtained by the condensation of ω-methoxyacetophenone with benzaldehyde, essentially as described (20). The details were modified and improved somewhat, but will be omitted here, because of the superiority of the new method. When carefully purified it could be kept

* *The preliminary work and establishing of optimum conditions for securing this previously known (1,22) ketone by the Grignard reaction were done by Mr. A. M. Irvin at Tufts College, Mass., in 1928-1929, under the senior author's direction. The details of the final purification and results in the Grignard machine (17) were determined in the present work.*

† *A carefully purified sample gave off gas with methylmagnesium iodide; quantitative measurement in the Grignard machine (17) showed 50.5% addition and 5.9% active hydrogen; cf. Ref. 4, in which gas evolution with nitriles of low molecular weight has been noted previously.*

in a cool place unchanged for several years; m.p. 34–35° C. The 2,4-dinitrophenylhydrazone crystallized from alcohol in bright orange needles, m.p. 210–211° C. Calcd. for $C_{22}H_{18}O_6N_4$: N, 13.4%. Found: N, 13.3%.

B. The Addition Reactions

2-Keto-3-cyano-5-methoxy-4,6-diphenyl-6-hydroxypiperidine, (*I*). A mixture of sodium methylate (from 1.4 gm. of sodium and 70 cc. of dry methanol), 7.5 gm. of cyanoacetamide, and 12 gm. of α-methoxybenzalacetophenone was left at 40° C. for a week; crystals usually appeared after a day or could be induced to appear by scratching with a rod. These were filtered and combined with a second crop isolated from the acidified filtrate; the combined yield was 55–60%. After repeated recrystallization from methanol the substance formed large translucent prisms, m.p. 241–242° C. It is insoluble in cold alcohol, acetone, ethyl acetate and chloroform, moderately soluble in hot alcohol, and readily soluble in acetic acid and pyridine. Calcd. for $C_{19}H_{18}O_3N_2$: C, 70.8; H, 5.6; N, 8.7%. Found: C, 70.8; H, 5.6; N, 8.7%.* Careful examination of the residues and solutions revealed only unchanged components and traces of the hydroxypyridine (V).

The addition product was recovered unchanged from all attempts to secure an oxime, semicarbazone or phenylhydrazone. It dissolved in cold, dilute sodium hydroxide, and was precipitated unchanged on acidification.

The other addends, listed in the introduction, were used in very dry and ordinary alcohol, at the boiling point and room temperature, and in the presence of a trace or excess of sodium methylate, as well as diethylamine and piperidine. There was no evidence of an addition product in any instance.

C. The Six-membered Series

3-Cyano-4,6-diphenyl-2,5,6-trimethoxytetrahydropyridine (*IV*). This was very easily obtained by treating a solution of the addition product in an excess of 10% sodium hydroxide with an excess of methyl sulphate. The product slowly separated; it was filtered after an hour, washed and recrystallized from methanol, in which it is sparingly soluble. It melted at 290° C. with sublimation. Calcd. for $C_{21}H_{22}O_3N_2$: N, 8.0, CH_3O, 26.6%; mol. wt., 350. Found: N, 7.9; CH_3O, 27.0%; mol. wt., 344.

2-Hydroxy-3-cyano-5-methoxy-4,6-diphenylpyridine (*V*). The best yield was obtained by refluxing for two hours a mixture of 20 cc. of benzene, 3 gm. of phosphorus pentachloride, and an equal weight of the addition product. It was then well mixed with water; the benzene layer was drawn off and evaporated to dryness. The residue was triturated with cold, dilute sodium hydroxide to remove unchanged starting material, thoroughly washed and dried; the yield was 1.6 gm. (55%). On recrystallization from pyridine it

* *The nitrogen analyses were done by both semi-micro Dumas (19) and Kjeldahl methods. Since the latter was frequently low, the method was investigated, improved, and the results published (24). Almost without exception a low percentage of nitrogen was found in the cyclic nitrogen compounds by the original Kjeldahl method (2). The methoxyl groups were determined by a semi-micro Zeisel method (6). While good results were obtained with the partially reduced compounds, the pyridines and piperidines usually gave low values. These discrepancies often furnished a valuable clue as to the nature of the substance being analyzed.*

formed greenish-yellow rods, m.p. 318–320° C. with decomposition. It is soluble in pyridine, chloroform and acetic acid, but insoluble in the other common solvents. Calcd. for $C_{19}H_{14}O_2N_2$: N, 9.3; CH_3O, 10.2%. Found: N, 9.4; CH_3O, 9.5%. The hydroxypyridine was also obtained in the following ways: in the preparation of the addition product; from the latter by the action of aluminium chloride, hydrogen chloride in methanol or chloroform, alcoholic potash, and bromine in chloroform; from the gummy residue obtained by bromination, after boiling with alcoholic potash; from the dihydropyridine (VI) and hydrogen chloride in chloroform.

The hydroxypyridine dissolves in hot 2% sodium hydroxide solution and a sodium salt separates on cooling; acidification regenerates the original substance. The sodium salt was not alkylated by methyl sulphate. It was unaffected by phosphorus pentoxide in boiling xylene, but was destroyed by hydrogen iodide, giving indeterminate products.

2-Keto-3-cyano-5-methoxy-4,6-diphenyltetrahydropyridine (VI). Dry hydrogen chloride was passed into a suspension of 5 gm. of the addition product in cold, dry chloroform for four hours. The flask was closed and left for four days; the solution was then washed free from acid by water, and the chloroform layer evaporated. The residue was separated with difficulty into the tetrahydro compound and the hydroxypyridine (V). It dissolved very slowly in dilute sodium hydroxide. The yield was 2 gm. (40%); it is insoluble in benzene, ether, and cold methanol, but soluble in chloroform, acetic acid, pyridine, and hot methanol, from which it separates in prisms, m.p. 233–235° C. Calcd. for $C_{19}H_{16}O_2N_2$: N, 9.2; OCH_3, 10.2%. Found: N, 8.8; CH_3O, 10.2%. A chloroform solution, saturated with hydrogen chloride and allowed to stand for four weeks, was partially transformed into the hydroxypyridine.

2-Keto-5-methoxy-4,6-diphenyl-6-hydroxypiperidine (IX). A benzene solution of 20 gm. of aluminium chloride and 10 gm. of addition product was refluxed for seven hours, poured into water, made strongly alkaline, and warmed to 40° C. The insoluble solid was filtered and extracted with methanol. On cooling, the piperidine derivative separated as needles, which, after purification, had a melting point of 155° C.; yield, 5%. Calcd. for $C_{18}H_{19}O_3N$: N, 4.7; CH_3O, 10.4%. Found: N, 4.6; CH_3O, 10.0%. The hydroxypyridine (V) was isolated from the alkaline solution by appropriate manipulations.

By a similar treatment of (XI), the corresponding tetrahydro- and hydroxypyridines were isolated from the large number of substances formed.

Action of Bromine. (a) In acetic acid. To a boiling mixture of 5 gm. of the addition product, 50 cc. of acetic acid, and a drop of acetone, was slowly added an acetic acid solution of bromine at such a rate as to maintain a deep red color in the mixture—this required four hours and used up two equivalents of bromine. On cooling, some ammonium bromide separated. The solution was poured into water and left for 15 hr., during which time a mass of pliable, greenish filaments, up to 2 in. in length, separated on the surface. These

were removed and dried; it was impossible to purify them, although they have been kept for two years apparently unchanged. The melting point was 125–130° C. with decomposition. Found: N, 6.3; Br, 14.7; CH_3O, 3.1%. When placed in methanol they crumbled and a brownish sediment was deposited; the solution left a brownish gum. The sediment was tediously separated into starting material, hydroxypyridine (V), the bromo compound (XII) (60% yield) and the bromine-free (XIV) (2–5% yield).

2-Keto-3-bromo-3-cyano-5-methoxy-4,6-diphenyl-6-hydroxytetrahydropyridine (XII), forms pale yellow needles, m.p. 292–293° C., insoluble in the Skelly-Solves, ether, alcohol, and benzene, but soluble in acetic acid and chloroform. Calcd. for $C_{19}H_{17}O_3N_2Br$: N, 7.0; Br, 19.9; CH_3O, 7.7%. Found: N, 7.2; Br, 19.5; CH_3O, 7.4%. It was unaffected by acetic anhydride (at 40° C.), phosphorus pentoxide in boiling xylene, and sulphuric acid; it gave some of the hydroxypyridine (V) when refluxed for three hours with acetyl chloride. It dissolved in dilute sodium hydroxide and was reprecipitated by mineral acids; it was recovered nearly quantitatively after 2.5 hr. refluxing with $N/2$ alcoholic potash.

2-Keto-5-methoxy-4,6-diphenyl-6-hydroxytetrahydropyridine (XIV), is insoluble in alkali and crystallizes from alcohol in tiny prisms, m.p. 252–253° C. Calcd. for $C_{18}H_{17}O_3N$: N, 4.7; CH_3O, 10.5%. Found: N, 4.6; CH_3O, 10.0%.

(b) In alkaline solution. To the sodium methylate prepared from 0.3 gm. of sodium and 100 cc. of methanol was added 4.2 gm. of the addition product, and at the boiling point, bromine added as fast as the violence of the action permitted (20 min.). After 24 hr. the solid that had separated was found to be unchanged material and the bromopiperidine (XII). On evaporation of the solution *in vacuo*, a new substance separated; after purification from methanol, it formed bright yellow needles, m.p. 149–150° C.; yield, 40%. Calcd. for $C_{19}H_{17}O_5N$: C, 67.4; H, 5.0; N, 4.1%. Found: C, 67.9; H, 4.7; N, 3.9%. 2-Keto-3-carboxy-5-methoxy-4,6-diphenyl-6-hydroxytetrahydropyridine dissolved readily in sodium bicarbonate solution and was reprecipitated by acids. When heated (180–200° C.) it lost carbon dioxide; on purification, the residue was the non-acidic (XIV).

Action of Acetic Anhydride. 2-Acetyl-5-methoxy-4,6-diphenyl-6-hydroxytetrahydropyridine (XV). A mixture of 2 gm. of the addition product, 125 cc. of acetic anhydride (smaller amounts led to very poor yields) and two drops of concentrated sulphuric acid (or 10 cc. of pyridine), was refluxed for two hours and worked up in the usual manner, decolorizing with Darco during the methanol recrystallization. It formed needles, very soluble in hot methanol (but almost insoluble at 10° C.), m.p. 126° C. Calcd. for $C_{20}H_{21}O_4N$: N, 4.1; CH_3O, 9.2; CH_3CO, 13.3%. Found: N, 4.4; CH_3O, 8.9; CH_3CO, 13.5%. On hydrolysis, and from the residual solution after determining the acetyl content by Perkin's method (7), 2-hydroxy-5-methoxy-4,6-diphenyldihydropyridine (XVI) was quantitatively obtained. It separated in cubes from the alcohols, m.p. 173–174° C. Calcd. for $C_{18}H_{17}O_2N$: N, 5.0; CH_3O, 11.1%. Found: N, 5.0; CH_3O, 10.8%.

D. The Five-membered Series

4-Phenyl-5-benzoylpyrrolidone-2 (XVIII). A mixture of 20 cc. of concentrated sulphuric acid and 10 cc. of water was cooled to 50–60° C., 1 gm. of the addition product added, and the mixture warmed on the steam bath for 3.5 hr. A precipitate was obtained on pouring the solution into water; this was dissolved in methanol and separated in long needles, m.p. 172° C. An isomeric form resulted on attempted esterification of the product by the use of alcoholic hydrogen chloride; it was found essential to keep the temperature low, otherwise only oils resulted. The solvent was removed in a vacuum desiccator, and needles, m.p. 153–154° C., slowly separated. Calcd. for $C_{17}H_{14}O_2N$: C, 77.0; H, 5.7; N, 5.3%. Found: (172° C.) C, 77.5; H, 5.5; N, 5.3%; (153° C.) N, 5.1%. Both gave the same 2,4-dinitrophenylhydrazone; the latter separated from alcohol in yellow needles, m.p. 205° C. The lactam was unaffected by phosphorus pentoxide in xylene, very slowly destroyed by sulphuric acid (as below), but rapidly converted into black, untractable oils by alkalies with evolution of ammonia.

3-Carbamyl-4-phenyl-5-benzoylpyrrolidone-2 (X). This was isolated as a by-product from the reaction between aluminium chloride and the addition product, by fractional precipitation from the alkaline solution. It was freed from aluminium by a repetition of the process, followed by recrystallization from hot methanol; the yield was 5–10%. It is sparingly soluble in cold methanol and pyridine; it formed prisms, m.p. 273° C., with effervescence. Calcd. for $C_{18}H_{16}O_3N_2$: C, 70.1; H, 5.2; N, 9.1%. Found: C, 70.7; H, 5.2; N, 9.2%. The 2,4-dinitrophenylhydrazone crystallized from alcohol in brown prisms, m.p. 280–281° C.

The gum obtained by the original treatment with sulphuric acid was dissolved in chloroform and extracted with chilled, dilute sodium hydroxide. The alkaline solution was immediately placed in a separatory funnel containing cold ether and dilute hydrochloric acid; after shaking, and working up the ether layer, a solid resulted, which was separated with difficulty into a mixture of lactones (XX), and hydroxyacid (XIX). The lactones were converted into 2,4-dinitrophenylhydrazones which were more easily separated, and found to be identical with those formed from the lactones, synthesized by Kohler's method (14). The hydroxyacid and hydroxyamide (XXI), gave a mixture of the same dinitrophenylhydrazones. The lactone, m.p. 130° C., gave the derivative, bright yellow plates, m.p. 164° C., but the low melting lactone (98° C.) gave an isomer; orange-yellow plates, m.p. 192° C. Calcd. for $C_{23}H_{17}O_6N_4$: C, 62.0; H, 3.8%. Found: C, 62.4; H, 4.0%.

When the gum was directly treated with 2,4-dinitrophenylhydrazine, it gave a mixture of three dinitrophenylhydrazones, two of which were those described above; the third formed needles from alcohol, m.p. 182° C. Found: N, 11.2, 11.0%; mol. wt., 510. This same dinitrophenylhydrazone was also formed from gum resulting from treatment of the lactones or lactam with sulphuric acid under the same conditions as were used with the addition product.

β-Phenyl-γ-benzoyl-γ-hydroxybutyramide (?) (XXI). A solution of the lactone in dry methanol was saturated with ammonia and allowed to stand at room temperature for several days. On evaporation, prisms, m.p. 173° C. (mixed melting point with (XVIII), 155–160° C.) resulted. Calcd. for $C_{17}H_{16}O_3N$: C, 72.4; H, 5.7%. Found: C, 72.5; H, 5.5%. When heated in a sealed tube, a small amount of (XXI) was formed, along with much black oil.

E. p-Nitrobenzyl Cyanide and α-Brombenzalacetophenone; Formation of a Mixture of 1-Cyano-1-p-nitrophenyl-2-benzoyl-3-phenylcyclopropanes

To a warm mixture of 5.7 gm. of the bromoketone, 3.2 gm. of the nitrile and 25 cc. of dry methanol was added, drop by drop, a solution of sodium methylate, until the color, at first green, and then magenta, had changed to a pure blue (the characteristic color given by the cyclopropane and alkalies). It was acidified with a few drops of acetic acid, and on scratching, the solid separated; yield, 2.5 gm. (48%). It was separated into a small amount of unused nitrile, and the two stereoisomeric cyclopropanes, m.p. 144° and 151° C. respectively. The residue was a green gum.

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REVIEWS AND NOTES

Laboratory Test of Ultrasonic Depth Sounder

Recently the National Research Laboratories were asked to test a Huson Ultrasonic Depth Sounder, of the type commonly installed on launches, and having a range of about 200 ft. Since it is believed this is the first time a depth sounder has been directly tested for such depths in a laboratory, it was thought worthwhile to record the results obtained.

The Huson sounder is made up of two identical magnetostriction rod oscillators, one used as transmitter, the other as receiver. The power used for driving the transmitter is derived from a motor-generator, operating at 400 volts. This voltage is used to charge a condenser, which discharges at periodic intervals through the coil wound around the rod oscillator. A damped oscillation is thus emitted into the water. The second rod picks up the echo, vibrates slightly, and hence induces a minute electric current in its surrounding coil. This current is amplified and eventually passes into a metal contact, moving rapidly up and down on a piece of paper, which rolls slowly past the contact. The paper has been made conducting by a potassium iodide solution, and as the current passes through it a discoloration occurs. The position of the drawn-out spot thus marked is determined by the distance the contact has travelled between the time of the transmission of the signal and the time of its reception, and hence is a measure of the distance the signal has travelled, *i.e.*, the depth of the sea-bottom. The spots on the paper occur close together and result in a line which is really a profile of the ocean floor over which the boat is moving.

To carry out the test, the basin used for testing models of aeroplane floats and ships' hulls was employed. This basin is 396 ft. long, 6 ft. deep and 9 ft. wide. Additional equipment in connection with the tank includes a light, metal carriage which can be moved up and down the length of the basin as required. On this carriage was mounted the gear used for sounding, the ultrasonic transmitter and receiver being clamped on a piece of angle iron, one above the other, so that they were immersed in the water, pointing horizontally, one about a foot above and the other about a foot below the mid-depth point. This was arranged so that one end of the tank would act as the reflector, the equivalent of various depths being obtained by moving the carriage to different distances from the end.

In an actual installation, the ultrasonic transmitter and receiver are placed inside the ship's hull, so that the energy must go and come through $\frac{1}{8}$ in. or more of metal. To simulate this condition, a large sheet of duralumin, $\frac{3}{8}$ in. thick, was hung from the carriage, in front of the transmitter and receiver.

The frequency of this sounder is only 16,000 cycles, so that a conical horn is used in connection with the rod oscillator to concentrate the energy into a beam. The formula for the half-angle of the beam is $\sin\frac{1}{2}\varphi = 1.2\ \lambda/D$,

where λ is the wave-length of the sound in the liquid (water), and D is the diameter of the transmitter. Since the aperture of the cone is only six inches, the above formula shows that the total angle of the beam is over 90°. With such a wide angle, it was feared that multiple reflections from the sides of the tank would cause a very indefinite echo, and that poorly defined depth measurements would most probably occur.

In Fig. 1 is shown a photograph of one of the records obtained. The ordinates represent depths; the abscissas, time intervals, or, in general, horizontal distances, the distances depending on the speed of the boat. Since this particular record was obtained as indicated above, the ordinates really represent distances from the end, or ends, of the tank. The abscissas, similarly, represent only time intervals.

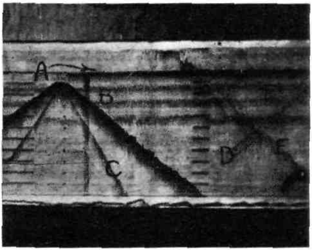

FIG. 1. *Photograph of record obtained with depth sounder.*

In the figure, the line marked "A" is the "zero" line. In an actual installation, it would indicate the surface of the water.

B is the line of depth, and was obtained by moving the carriage at a constant speed, first, towards one end, and later, away from it. Where B is parallel to the zero line, the carriage was not moving. As the "depth" increased, it will be noticed that the line became somewhat irregular. This presumably was caused by reflections from the tank walls.

Line C is the double depth line, caused by the sound being reflected from the end of the tank, then from the metal in front of the transmitter, again from the end of the tank, until finally the beam reached the receiver. This line often appears in practical sounding, on account of reflections from the surface of the water.

Line D was caused by the sound being reflected from the metal directly in front of the transmitter to the opposite end of the tank, back to the metal, and into the receiver. This line has a slope opposite to lines B and C, because the end of the tank from which its reflections occurred was approached (this "depth" became less) as the carriage receded from the main reflecting end.

Line E was caused by reflection from the first end, plus an additional reflection from each end. The beam of sound responsible for it had a path length of twice the distance from the transmitter to the reflecting end of the tank, plus twice the total length of the basin. Hence line E represents depths of 400 to 600 ft.

The results here recorded have shown that, in spite of some previous misgivings, the testing of a depth sounder in a narrow tank is quite feasible,

even up to equivalent depths of 400 ft. Some slight irregularities in the depth line were apparently caused by reflections from the walls of the basin, but aside from that, such reflections were of negligible effect.

In conclusion the writer would like to thank Mr. K. F. Tupper, in charge of the water basin, and Captain C. B. Potts for assistance rendered in connection with this test.

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RAPID DETERMINATION OF MOISTURE IN GRAIN

II. CALIBRATION AND COMPARISON OF ELECTRICAL MOISTURE METERS WITH VACUUM OVEN FOR HARD RED SPRING WHEAT[1]

By W. H. Cook[2], J. W. Hopkins[3] and W. F. Geddes[4]

Abstract

The electrical moisture meters studied were: the Burton-Pitt, Davies, Limbrick, hand-operated and motor-operated Tag-Heppenstall. The Burton-Pitt is a "dielectric" type; all the others depend on the resistance principle. The calibration curve for the Burton-Pitt instrument is parabolic within the range 11.0 to 17.0% moisture. The resistance type instruments all show a linear relation between moisture content and the logarithm of the resistance at moisture contents below 17.0%, and a parabolic relation at higher moisture contents. The accuracy of the meters, as determined by the experimental errors and the standard error of prediction of the vacuum oven moisture, decreases in the following order within the moisture range, 11.0 to 17.0%: motor-operated Tag-Heppenstall, Limbrick, hand-operated Tag-Heppenstall, Burton-Pitt and Davies. At higher moisture contents than 17.0% the Burton-Pitt and Davies instruments are definitely unreliable, and the remaining meters are also less precise.

The temperature coefficient of the wheat-instrument system was found to be appreciably different from that of the wheat alone in the Limbrick and Burton-Pitt. The temperature coefficient was also found to differ somewhat in different moisture ranges. The effect of humidity is most pronounced in the Limbrick, but is in all instruments small in comparison with that of temperature. The limited number of low grade samples studied appeared to follow the same calibration curve as high grade samples in all meters and 28 samples of the hard-kernelled variety Garnet were also found to follow the calibration curve deduced from the general reliability experiments in all meters, with the exception of the hand-operated Tag-Heppenstall. The discrepancy with this meter is not great and can be attributed to the roll space being unsuitable for this hard-kernelled variety.

The moisture range satisfactorily covered by the instruments decreases in the order: motor-operated Tag-Heppenstall, hand-operated Tag-Heppenstall, Limbrick, Burton-Pitt and Davies. In all cases the upper limit is finally determined by the increasing inaccuracy of the meter, rather than by its physical range, which is limited at 21.5% moisture with the Limbrick only when used at the highest sensitivity. With wheat below 11.0% moisture, only the Burton-Pitt and Tag-Heppenstall instruments can be used, and the calibration curve for the Burton-Pitt obtained with wheat of higher moisture content cannot be extrapolated over this lower range.

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1. Introduction

This investigation was undertaken to calibrate and to determine the general utility and accuracy of five electrical moisture testers, namely the Burton-Pitt, Davies, Limbrick and the hand-operated and motor-operated Tag-Heppenstall instruments, when used with hard red spring wheat. The investigation was planned to include a statistical study of the errors of method and observation, sampling error, and daily variations, as already outlined (5) in connection with the results obtained from the same series of wheat samples by the vacuum oven, 130° C. air oven and Brown-Duvel methods.

The meters investigated did not include all the kinds manufactured, the D-K (Schnellwasserbestimmer) and the dielectrometer (moistometer), both well known, being omitted as they were not available at the time this investigation was undertaken. Coleman (4) however, investigated these meters, which operate on a capacity principle, and found them inferior to the Tag-Heppenstall, a resistance type. The dielectric or capacity type was represented in this investigation by the Burton-Pitt. Some preliminary experiments on three of the meters included in the present study have been reported by Birchard (1).

2. Design and Operation of Instruments

Electrical moisture meters may be divided into two general types according to the principle employed. In the so-called "dielectric" type the grain is placed inside the windings of an induction coil or between the plates of a condenser forming part of a high-frequency oscillating circuit. The other type measures the electrical resistance of the grain, using direct current from a battery, and special electrodes.

In the Burton-Pitt dielectric instrument the circuit is so arranged that the direct current component of the plate current is altered by the introduction of the sample, and the accuracy of this class of instrument is therefore dependent on this change being closely related to the moisture content of the material, and on the stability of the oscillating circuit. The work of Burton and Pitt (2) suggests that the results obtained are dependent to some extent on the electrical conductivity, as well as the dielectric properties of the material under test.

The electrical resistance type makes use of the relationship between electrical resistance and moisture content observed in materials which contain soluble salts and relatively small amounts of water. As the electrical resistance of grain is quite high at all ordinary moisture contents, either a sensitive galvanometer, a microammeter, or vacuum tube amplification must be employed for the measurement of the transmitted current. Various types of electrodes are employed in the different makes of instruments and these naturally affect the magnitude of the observed resistances.

Burton-Pitt Tester

Burton and Pitt (3) describe the Burton-Pitt tester and give the results obtained from a series of determinations. In these the wheat was placed in a standard test tube, without weighing, and the tube inserted in the induction coils of the instrument. In the instrument provided for the present investigation, however, the grain is placed between the plates of a condenser, contained in a test cell connected to the instrument by means of flexible cord, an arrangement they presumably found to give more satisfactory results. The instrument as originally received was fitted with a 201A vacuum tube, but on the recommendation of Prof. Burton this was replaced by a 112A tube which was found to give more consistent readings. The sensitivity employed in these tests was a milliammeter deflection of 90 when the constant capacity condenser was in the circuit. The setting of the zero point and the sensitivity were checked before each test and minor adjustments were frequently found necessary.

After making the above adjustments with the empty test box connected to the instrument, the actual test consisted merely of weighing out the desired amount of grain, pouring it into the test box and reading the milliammeter. As the instrument settings are sometimes altered slightly by moving the cell, such movements should be avoided. For the purpose of the present tests the cell was mounted in a wooden block having a wooden slide which fitted flush with the bottom; drawing this slide discharged the grain after the test. The cell held 100 gm. of wheat of relatively low bushel weight, so this quantity, weighed to within ± 0.1 gm. was employed in the test. The wheat was poured in uniformly without packing.

This meter can hardly be regarded as a readily portable instrument, for although the meter unit proper is not large, the battery box, containing a storage cell and rectifier in addition to the dry batteries, is large and heavy. Moreover, if the instrument is to be used for making any number of tests it must be operated near a source of electric current in order to charge the storage cell. About 6 sq. ft. of table space is required for operation in addition to a smaller space beneath the table to accommodate the batteries. The only accessory equipment required is a balance for weighing the grain. The time required to make a test is discussed, in relation to the other meters, toward the end of this section but it should be noted that the instrument must be connected up from 20 to 30 minutes before it is to be used in order to allow the tube to warm up and become stable.

Davies Moisture Meter

The Davies moisture meter operates on the resistance principle and has been described by the inventor (6). It was the simplest instrument of this type tested, consisting only of a test-cell, sensitive galvanometer, standardizing and protecting resistances, switch, and battery, all of which are contained in a compact carrying case. The protecting resistance is permanently in series with the galvanometer, and when the switch is turned to the standard-

izing position the galvanometer deflection gives a measure of the battery voltage, which is adjustable in increments by means of wander-plugs fitting into holes in the battery.

In preliminary work it was found that the instrument was not sufficiently sensitive to give a deflection of measurable magnitude with "straight" grains. As the galvanometer gave a full scale deflection at 46.86 volts, a voltmeter of high resistance was connected to the instrument, and readings taken at 100 volts on all samples giving small deflections at 46.86 volts. A further modification consisted of placing a 10,000-ohm potentiometer in the battery circuit so that the voltage could be varied continuously, it having been found that adjustments by means of the wander-plugs could not be made closely enough for accurate work. Preliminary tests were made using both ground and whole wheat, as the instructions did not state which was to be used. The ground samples gave somewhat greater deflections but this increase in sensitivity was not sufficient to give significantly higher readings with dry grain. The actual tests of this instrument were therefore made with whole wheat.

The Davies meter was the smallest and most readily portable instrument of those tested, and required only about 2 sq. ft. of space for its operation. The instrument is entirely self-contained and no preliminary grinding or weighing of the sample is necessary. The grain, however, must be packed in the test cell by hand, using a heavy plunger. The electrodes are placed in the bottom and on the sides of the test cell, and it is asserted that the reading is independent of the amount of grain, provided the depth of the sample, after packing, exceeds 3 cm. Although Davies (6) has shown that some form of polarization occurs in these test cells he feels that this does not vitiate the method as long as a constant voltage is used for calibration and testing. No attempt was made to study the effect of polarization in this investigation, but the experiments were arranged so that a sufficient number of samples would be tested at each voltage employed to provide a reliable calibration curve.

Limbrick Moisture Tester

The Limbrick moisture tester (8) also operates on the resistance principle, the resistance of the sample being compared with a resistance bank which is adjustable by increments. Vacuum tube amplification is employed in conjunction with a milliammeter for measuring the current. In order to overcome any error arising from aging or other change in the vacuum tube, the deflection of the milliammeter is observed while a push button which places the grain only in the circuit is depressed. Another push button is then depressed which places the resistance bank only in the circuit and the resistance is then adjusted to give the same deflection as the grain. Adjustments are provided for setting the meter at the desired sensitivity. The instrument studied was the 20A, the larger and more accurate of the two models made.

Before a test can be made, the grain must be ground in a small hand mill supplied with the instrument. The burrs in this mill are set to grind the

grain rather coarsely, adjustment being made at the factory. The grain is not weighed, but a constant volume of the ground material is measured out in a measuring cup and introduced into the test cell through a small hopper. The meter unit proper is somewhat larger and heavier than the Davies, but apart from the grinder, it is self-contained. Only a few minutes are required for the amplifying tube to warm up before a test can be made. Ordinarily this instrument, including the grinder, requires about 4 sq. ft. of table space.

Tag-Heppenstall Meters

The hand-operated Tag-Heppenstall moisture meter has already been described by Coleman (4) and by Geddes and Winkler (7). Both hand- and motor-operated models consist of two distinct parts, the roller electrodes and the meter unit for measuring the current. The latter consists of an Ayrton shunt, a sensitive microammeter, batteries, and a resistance for adjusting the current. The electrodes consist of two corrugated rollers. Although the meter unit is the same for both hand- and motor-operated electrodes, the electrodes themselves differ considerably, those of the motor-operated model being much larger. The hand-operated model is easily portable, the roller electrodes being somewhat heavier than the Limbrick grinder, but the instrument itself less bulky. No external source of electric current is required. The electrodes of the motor-operated model are much heavier and as current is required to operate the motor this can scarcely be regarded as a thoroughly portable instrument. The hand model requires about 4 sq. ft. of table space and the motor model slightly more.

The roller electrodes appear to be superior to the test cells with static electrodes used in the other resistance-type instruments. They are rapid in operation (see Table I), and no grinding, weighing or measurement is required. Although no attempt was made to determine the extent to which polarization affects the results obtained with the different meters, it seems likely that this effect had the least influence in the Tag-Heppenstall meters, since both the electrodes and sample are in continuous motion throughout the test. Another advantage of the roller electrodes is that impurities in the grain, such as a piece of metal, will cause the needle to jump but will not affect the "average" reading obtained, whereas in test cells such foreign matter will undoubtedly affect the resistance, and consequently the apparent moisture content. The observed resistances with the roller electrodes are also much lower than those to be measured in the test cells of the Limbrick and Davies meters.

Time of Operation

Although these instruments are all classed as rapid testers, there was a considerable difference in the time required to operate the different makes. The average time required for making duplicate tests, by the two operators allotted at random to the instruments during the general reliability test, was noted and is recorded in Table I. The reported times cover only the actual period required for manipulating the instrument and accessories

(grinder and balance where required) and do not include the time consumed in selecting samples, making interpolations, or reading conversion charts, etc. The motor-operated Tag-Heppenstall was found to be the most rapid of the testers investigated, while the hand-operated model was only slightly slower. Although frequent adjustments are necessary in operating the Burton-Pitt the actual operation of the meter is usually not as time-consuming as the preliminary weighing of the sample. The Davies instrument was found to be relatively slow. The sensitivity had to be changed frequently, and as the galvanometer pointer was not damped properly, some time was required for it to come to rest. The Limbrick was the slowest, partly because the grain had to be ground but chiefly because the manufacturer's instructions required three independent grindings and readings to be taken on each sample. According to these instructions the first of these readings is not to be used, since it might be in error due to moisture remaining in the cell from the previous sample, etc. In order to determine whether or not this first reading is less reliable than the other two, the deviations of the first trial from the means of Trials 2 and 3 were compared with the variance of Trials 2 and 3 individually. This was done on 120 samples distributed throughout the entire moisture range, and there was no significant difference in these variances, suggesting that the first reading need not be considered less trustworthy than the other two. The time of operation, as given in Table I, can therefore be reduced to about two-thirds of the reported value, or 3.6 min. for a duplicate test. Even so, this meter is still one of the slowest in operation.

TABLE I

RAPIDITY OF DETERMINATION OF MOISTURE BY ELECTRICAL METERS

Meter	Average time for duplicate determinations (*min.*)
Tag-Heppenstall (motor)	1.2
Tag-Heppenstall (hand)	1.5
Burton-Pitt	2.4
Davies	3.7
Limbrick	5.4

3. Experimental Details

All the instruments were operated in accordance with the instructions supplied by the manufacturers, and with minor exceptions no effort was made to determine the best technique, operating conditions, or settings employed. The instruments were examined and tested thoroughly in the Physics Division of the National Research Laboratories before and after the investigation to insure that none was defective during the tests. The resistances corresponding to different meter readings, for all instruments operating on the resistance principle, were determined prior to the actual tests, so that the moisture values could be plotted as a function of the resistance in making the calibration charts. It was felt that a calibration chart showing the relationship between resistance and moisture content would be of more general utility than one showing the moisture content at various meter readings, since the latter would be applicable only to particular instruments. To adjust the

resistances on each instrument accurately would be more difficult than measuring the actual value. If this value is known the data obtained in this investigation should be applicable to the calibration of any instrument of a particular make, provided the test cell is of the same construction and dimensions throughout.

In the general reliability tests, two operators read the five meters. To avoid any systematic error due to personal factors, a separate random allotment of meters and operators was made for each sample. In determining the temperature and humidity coefficients, one operator read all the instruments. Before any of the tests were undertaken the operators, who were skilled laboratory technicians, were trained for several days in the use of all the instruments.

It was planned originally to make all of the meter tests in a room maintained at a constant temperature of 72° F. and a constant relative humidity of 40%. Owing to the number of men in the room however, the temperature frequently rose to 75° F., with the result that it was necessary to apply temperature corrections to the majority of the observed instrumental readings. These changes in temperature also caused some fluctuation in the relative humidity, but it will be shown later that these small variations had no significant effect on the results. In the experiments on the effect of temperature and humidity only one man worked in the room, and the required conditions were maintained within $\pm\frac{1}{2}$° F. and ± 2% relative humidity.

The temperature of the grain was measured in all experiments, and since all samples had been stored in the testing room for 24–36 hr. their temperature seldom differed from that of the room and instrument by more than $\frac{1}{2}$° F. When such differences occurred, the temperature of the grain was employed in making subsequent calculations. As only one of the meters, the Limbrick, is provided with a thermometer in the test cell, the temperature of the grain used in the other instruments was determined just before it was tested. Since the temperature of the room and the grain were practically the same, only 30–60 sec. were allowed for the thermometer to reach equilibrium before the temperature reading was taken. Grinding the grain for the Limbrick usually raised its temperature 2–3° F. above that of the room, and in this case the temperature was read 30 sec. after placing the grain in the cell, as recommended by the manufacturer.

As previously described (5, § 2) the wheat samples employed were allowed to stand for one month in moisture-tight cans before testing. Under these circumstances a reasonably uniform distribution of moisture throughout the entire sample is to be expected. Grain freshly sampled from a car or bin may, however, be more heterogeneous, in which case, as suggested by Birchard (1, p. 16), less accurate results may be obtained.

4. Effect of Temperature

It is well known that the electrical resistance of any substance varies with the temperature, and in consequence the reading obtained from an instrument

of the resistance type will depend on the temperature at which the test is made as well as on the moisture content of the grain. Coleman (4) has shown that the temperature coefficient of grain is so high that fluctuations of even a few degrees will affect the results appreciably. An accurate knowledge of the temperature coefficient is therefore essential if precise results are to be obtained. There appears to be some diversity of opinion as to whether temperature corrections are necessary with the dielectric type of meter. Burton and Pitt (3) state that the readings obtained with their instrument are independent of the temperature and humidity of the room in which it is used. *A priori*, a temperature effect is to be expected regardless of whether the quantity observed is dependent on the dielectric constant of water or the conductivity of the material, since both of these properties are known to be affected by temperature. Coleman has pointed out that instruments of the dielectric type do not give the same readings with grain of different temperatures, and that the temperature coefficient of the dielectric constant of water does not account for the whole of the observed effect; he suggests also that the temperature coefficient of these instruments may not be linear through a range of moisture content.

In commercial testing two kinds of temperature variation may arise. The instrument and grain to be tested may be at the same temperature, *i.e.*, the temperature of the test room, in which case the temperature effect will depend on the combined temperature coefficient of the grain-instrument system; or the grain, freshly collected from a car or bin, may have a much higher or lower temperature than that of the instrument, the additional effect in this case depending on the temperature coefficient of the grain only.

These two temperature coefficients, and the humidity coefficient, were determined for thirty samples of wheat of different grade and moisture content, as shown in Table II; no artificially dampened samples were employed. Each sample was cleaned and subdivided into 56 subsamples using the riffle sampler and method previously described (5). These were allotted to the different instruments and conditions of test at random, five sets of subsamples being used for the vacuum oven determination. The results obtained by this method appear in the table, and the variations observed between different trials are doubtless, judging from the work already reported, due chiefly to sampling errors. It was found during the reliability tests, to be described later, that the Davies meter was of little value for estimating the moisture content of wheat, and therefore no data were obtained regarding the effect of temperature and humidity on the readings of this meter.

In determining the temperature coefficient of the wheat only, the instruments were kept at a temperature of 72° F. $\pm\frac{1}{2}$° F. and a relative humidity of 30% $\pm$ 2%, and the grain was adjusted to temperatures of 0° F., 32° F., 72° F. and 100° F., by means of a thermostat where necessary. This wide temperature range was employed partly because it was felt that under certain climatic conditions such extremes in grain temperature might occasionally be

TABLE II

GRADE AND VACUUM OVEN MOISTURE CONTENT OF WHEAT SAMPLES USED IN STUDYING EFFECT OF TEMPERATURE AND HUMIDITY ON MOISTURE METER RESULTS

Sample No.	Grade	Percentage moisture by vacuum oven					
		Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Average
WS1-E	1 Northern	12.28	12.30	12.28	12.30	12.24	12.28
WSH-2A	1 Hard	13.16	13.10	13.08	13.10	13.04	13.10
WS1-D	1 Northern	13.16	13.16	13.10	13.06	13.12	13.12
WS1-C	1 Northern	13.50	13.50	13.52	13.50	13.54	13.53
WS1-B	1 Northern	13.94	14.08	14.01	14.02	13.94	14.00
WS2-A	2 Northern	14.68	14.69	14.58	14.54	14.58	14.61
WS2-B	2 Northern	14.20	14.34	14.30	14.30	14.28	14.28
WS2-C	2 Northern	14.38	14.36	14.32	14.20	14.34	14.32
WS1-A	1 Northern	14.48	14.52	14.46	14.26	14.44	14.43
WS2-D	2 Northern	15.48	15.58	15.57	15.53	15.58	15.55
WT1-A	Tough 1 Nor.	14.60	14.46	14.74	14.62	14.74	14.63
WT2-B	Tough 2 Nor.	15.39	15.48	15.41	15.34	15.37	15.40
WT4-A	Tough 4 Nor.	15.26	15.32	15.32	15.28	15.32	15.30
WT2-C	Tough 2 Nor.	15.64	15.66	15.72	15.46	15.78	15.65
WT5-B	Tough 5	15.53	15.50	15.56	15.37	15.50	15.49
WT2-D	Tough 2 Nor.	—	15.56	15.63	15.42	15.70	15.58
WT4-B	Tough 4 Nor.	15.80	15.82	15.92	15.77	15.98	15.86
WT4-C	Tough 4 Nor.	16.54	16.54	16.40	16.38	16.50	16.47
WT5-A	Tough 5	16.04	16.23	16.38	16.24	16.26	16.23
WT3-A	Tough 3 Nor.	16.57	16.70	16.86	16.50	16.40	16.61
WD4-F	Damp 4 Nor.	17.00	16.98	16.86	16.69	16.82	16.87
WDF-A	Damp Feed	17.51	17.56	17.58	17.61	17.66	17.58
WD6-C	Damp 6	18.77	18.77	18.68	18.61	18.69	18.70
WD3-C	Damp 3 Nor.	19.56	19.38	19.64	19.30	19.45	19.47
WD4-E	Damp 4 Nor.	19.16	19.14	19.08	18.95	19.00	19.07
WD2-A	Damp 2 Nor.	19.35	19.45	19.32	19.54	19.39	19.41
WD5-A	Damp 5	20.25	20.52	20.52	20.49	20.60	20.48
WD3-B	Damp 3 Nor.	21.70	21.68	21.44	21.78	21.78	21.68
WD4-A	Damp 4 Nor.	19.03	18.87	18.94	18.96	18.98	18.96
WD4-D	Damp 4 Nor.	21.09	21.04	20.88	21.00	20.99	21.00

experienced, and partly because it seemed desirable to obtain qualitative information as to whether the temperature effect was linear over this wide range. The hand-operated Tag-Heppenstall instrument was not included in this experiment.

It was found impossible to maintain the grain at exactly the above temperatures, even with the precaution of wrapping the cans, as removed from the thermostat, in a heavy towel. The actual temperature of the grain at the time of making the test was therefore determined. Some difficulty was experienced here as a considerable period is required for a thermometer to reach equilibrium in such material. The practice was finally adopted of transferring the thermometer rapidly from one sample to the next and allowing it to come to equilibrium in the latter while the former was being tested. The greatest source of error, however, arose from the fact that cold grain tended to cool the electrodes and test cells, and *vice versa*. In consequence the meter readings on any one sample showed a drift with time, and a similar effect may also have operated from sample to sample since the first ones tested tended to cool or warm the electrodes and decrease the temperature

differential in subsequent tests. The effect of the temperature drift observed in the individual samples was eliminated as far as possible by taking a meter reading as soon as it was practicable after the grain was introduced. As a second reading taken later was invariably different, only the first was employed in the graphs and calculations. The extent to which the cooling of the electrodes by one sample affected the reading obtained on the next sample could not be definitely determined, and although it seems probable that this effect was small, the experiment as a whole cannot be considered as of great precision.

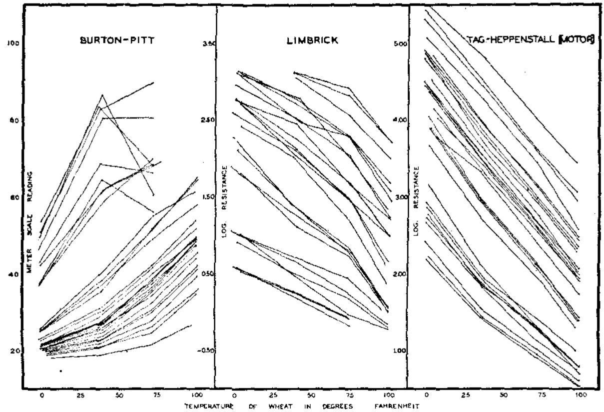

FIG. 1. *Effect of temperature of wheat on meter reading.*

The results obtained are shown in Fig. 1, in which the readings and logarithms of the resistance obtained at different temperatures in the Burton-Pitt and resistance instruments respectively, are plotted against the temperature. Readings obtained on the Burton-Pitt instrument are exceedingly erratic when the deflection exceeds 50 scale divisions. With this instrument the relation between temperature and meter readings appears to be curvilinear with samples of low moisture content, the curve tending to become straighter with greater slope as the moisture content increases.

Since the resistance of grain decreases with temperature the resistance type instruments have a positive coefficient in terms of moisture. The logarithm of the resistance, rather than the resistance itself, is employed as the former was found to be more directly proportional to the moisture content. Owing to differences in design, the resistances to be measured in the Limbrick are much greater than those encountered in the Tag-Heppenstall instruments. In the former case, therefore, the megohm, and in the latter

1,000 ohms, were selected as the units of resistance. It is difficult to say whether the temperature effect with the Limbrick is linear or curvilinear, but owing to the evident irregularity only a straight line was fitted to the points. The motor-operated Tag-Heppenstall results indicate that for the most part the temperature coefficient is linear throughout the temperature range studied.

The approximate temperature coefficient of each sample was determined graphically, the average values for the straight, tough, and damp classes being shown in Table III. It is evident that the temperature coefficient of wheat in the Burton-Pitt instrument is positive and also that it increases with the moisture content for straight and tough grain. The results obtained with damp wheat were erratic and the form of the curves in Fig. 1 suggests that different coefficients are applicable to different temperature ranges. The temperature coefficient in the resistance instruments, in terms of resistance, is negative and appears to be greatest at the intermediate resistances, *i.e.*, with tough grain. The magnitude of the difference between the three moisture classes is greater with the Limbrick than with the Tag-Heppenstall meter. The interpretation of these coefficients will be deferred until the moisture content–resistance relationships have been established in a later section of this paper.

TABLE III

TEMPERATURE COEFFICIENT OF WHEAT IN MOISTURE METERS (METERS AT CONSTANT TEMPERATURE)

Meter	Temperature coefficient per ° F.			Unit of measurement
	Straight*	Tough*	Damp*	
Burton-Pitt	0.21	0.31	0.72[1]; −0.10[2]	Meter scale division
Limbrick	−0.0160	−0.0203	−0.0121	Logarithm of resistance in megohms
Tag-Heppenstall (motor)	−0.0241	−0.0244	−0.0200	Logarithm of resistance in 1000's of ohms

**For exact definition of moisture limits see Table I, previous paper (5).*
[1] *0–32° F.* [2] *32–72° F.*

The determination of the temperature coefficient of the grain–instrument system was effected with greater precision owing to the elimination of the error arising from differential temperature conditions. Further subsamples of the thirty samples referred to were employed for this purpose. A series of six temperatures covering the range ordinarily experienced in a room, namely, 60, 66, 72, 78, 84, and 90° F., was employed. The actual temperature seldom varied more than $\pm\frac{1}{2}°$ F. from the desired value, and where there was a slight variation between the temperature of the grain and the room, the temperature of the grain was used in subsequent calculations. The relative humidity was maintained at $30\% \pm 2\%$ at all the temperatures employed in this experiment. It was recognized that this constant relative humidity did not represent the

same vapor tension, but it was felt that a fixed relative humidity at the different temperatures was a better approximation of the conditions obtaining in practice than either a fixed absolute humidity or a fixed vapor tension deficit.

The results obtained are plotted in Fig. 2. For the most part the ordinary deviations from a linear relationship between meter reading and temperature can be attributed to sampling and experimental errors. The Burton-Pitt, however, gave erratic results with nearly all of the samples containing sufficient moisture to result in deflections exceeding 50 to 60 scale divisions, and it appears therefore that this is due to some inherent fault in the instrument. There are two points that are seriously out of line in the motor-operated Tag-Heppenstall chart, but these are believed to be due to some mistake on the part of the operator.

Examination of Fig. 2 suggests that the temperature coefficient varies with the moisture content of the grain. In order to determine the temperature coefficient, a straight line was fitted to the points obtained with each

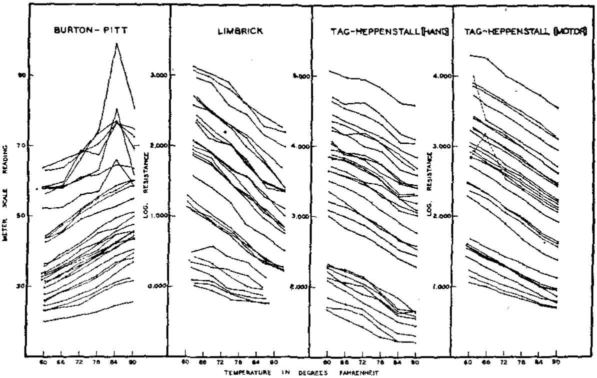

FIG. 2. *Effect of temperature of wheat—instrument system on meter reading.*

sample by the method of Least Squares. Since the high moisture samples gave erratic results in the Burton-Pitt, especially at temperatures above 78° F., the straight line was fitted only at intermediate temperatures in the neighborhood of 72° F. In Fig. 3, the slope of the line, representing the temperature coefficient, is shown plotted against the meter reading or logarithm of the resistance of the sample at 72° F. which is proportional to the moisture content. As the temperature of the grain tested in the Limbrick instrument was always increased by grinding, these results are computed to a basis of 75° F. It is evident from the figure that the temperature coefficient

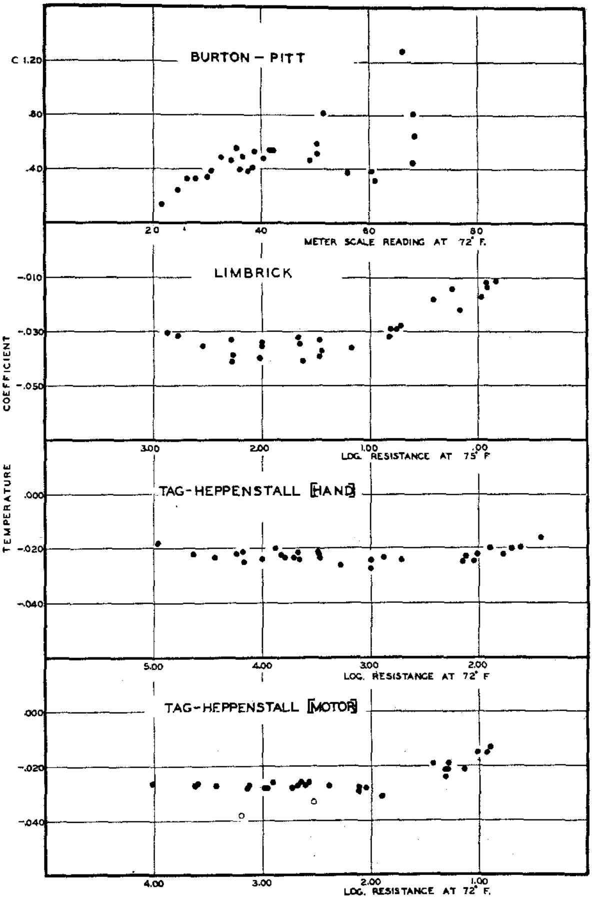

FIG. 3. *Variation of temperature coefficient of wheat—instrument system with moisture content.*

is dependent on the moisture content. The coefficient is positive for the Burton-Pitt, increasing with the meter reading or moisture content of the sample. With the resistance instruments the temperature coefficient in terms of resistance is again negative and the tendency is for this coefficient to increase in absolute magnitude with the resistance, *i.e.*, decrease as the moisture content increases.

Since the temperature coefficient depends on the moisture content of the grain, the total range of the meter readings was divided into smaller ranges over which a common temperature correction might be applied. These ranges, together with the average temperature coefficient applicable to each and the standard error of these average values, are given in Table IV. These values were employed in making subsequent corrections, in connection with

TABLE IV

TEMPERATURE COEFFICIENT OF WHEAT–INSTRUMENT SYSTEM (WHEAT AND METER AT SAME TEMPERATURE)

Meter	Meter range	Temp. coeff. per ° F.	Standard error	Unit of measurement
Burton-Pitt	20–30	0.26	0.04	Meter scale division
	30–50	0.46	0.02	
	50–60	0.58	0.09	
	Over 60	0.72	0.14	
Limbrick	3.00–2.50	−0.0323	0.0014	Logarithm of resistance in megohms
	2.50–1.00	−0.0364	0.0008	
	1.00–0.50	−0.0296	0.0008	
	Under 0.50	−0.0153	0.0014	
Tag-Heppenstall (hand)	5.00–2.00	−0.0228	0.0004	Logarithm of resistance in 1000's of ohms
	2.00–1.50	−0.0205	0.0006	
	Under 1.50	−0.0159	0.0013	
Tag-Heppenstall (motor)	4.00–1.50	−0.0274	0.0003	Logarithm of resistance in 1000's of ohms
	1.50–1.00	−0.0202	0.0010	
	Under 1.00	−0.0141	0.0011	

the general reliability tests, for the variations which occurred in the room temperature, since under these conditions the temperature of both the grain and the instruments is affected.

Although the results presented in Table III are, for reasons already given, less precise than those in Table IV, a comparison of these two tables gives some idea of the difference between the temperature coefficient of the wheat only, and that of the instrument and wheat together. The data in the two tables are not averaged over exactly the same moisture ranges, but this is of little consequence compared with the experimental errors in the first experiment. With the Burton-Pitt, within the moisture range of straight and tough wheat where the instrument is reasonably accurate, the coefficient of the wheat only is less than the combined coefficient of the wheat and the instrument, indicating that the meter itself is affected by temperature even

after it has been calibrated and set at the correct sensitivity at each temperature. With the Limbrick, the temperature coefficient of the wheat is on the average only about two-thirds of the combined temperature coefficient. This suggests that the resistances in the instrument also change with temperature, but in the opposite direction, *i.e.*, increasing with temperature, whereas the resistance of the wheat decreases with temperature. Whether or not the difference between the two temperature coefficients can be attributed entirely to the resistance coils is not known. A measurement of the temperature coefficient of a 1-megohm "metallized" type of resistor, similar to those used in this instrument, showed that it was much larger than that of wire-wound resistances, but was still too small to account for the magnitude of the observed effect. It is possible, however, that the temperature coefficient of the metallized resistor increases with the resistance and, since resistances as high as 200 megohms are used, this might account for the temperature coefficient observed. The two temperature coefficients determined for the motor-operated Tag-Heppenstall instrument are essentially the same within experimental error. This might be expected, partly because of the circuit employed, and partly because the resistances are made of manganin, which has an extremely small temperature coefficient.

5. Effect of Humidity

Nothing appears to be known concerning the influence of humidity on these electrical moisture testers. Long exposure of the grain to atmospheres of high or low humidity would naturally change its moisture content, but short exposures to the humidity of the test room should not have any significant effect. Any influence of variable humidity while testing must therefore be attributed to an effect on the instrument. With a meter of the resistance type it is possible that high humidity might decrease the resistance of the surface leakage path over the bakelite or other insulating material between the electrodes. This is especially true when the observed resistances are high, as in the Limbrick. A similar effect is possible but less likely in the dielectric instrument, but even here the humidity is likely to affect the air condenser and the surface of the test condenser. This interfering influence may, however, be removed by making the necessary adjustments of the calibration and sensitivity.

In order to determine the effect of humidity experimentally, further portions of the samples employed in the temperature experiments were tested in the Burton-Pitt, Limbrick, and motor-operated Tag-Heppenstall instruments. Relative humidities of 20, 30, 40, 50, 60 and 70% at a temperature of 72° F. were employed, and the results are shown in Fig. 4. Those obtained with the Limbrick apply at 75° F., since the grain is warmed somewhat by grinding. In all cases any effect due to small variations in temperature was removed by applying the temperature corrections already discussed.

It is apparent from Fig. 4 that the effect of humidity is small compared with that of temperature. Close examination also shows that the effect varies somewhat with the moisture content of the wheat, and in order to study this more exactly the approximate humidity coefficient was determined graphically for each sample and averaged separately for the "straight", "tough", and "damp" moisture classes. The results for all three meters are shown in Table V.

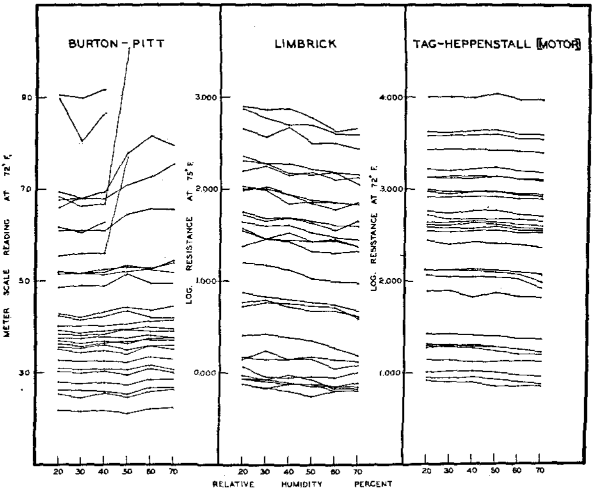

FIG. 4. *Effect of humidity on meter reading.*

TABLE V

HUMIDITY COEFFICIENT OF WHEAT–INSTRUMENT SYSTEM (WHEAT AND METER AT SAME HUMIDITY)

Meter	Humidity coefficient per 1% change in relative humidity at 72° F.			Unit of measurement
	Straight*	Tough*	Damp*	
Burton-Pitt	0.0089	0.0137	0.1782	Meter scale division
Limbrick	−0.00356	−0.00331	−0.00198	Logarithm of resistance in megohms
Tag-Heppenstall (motor)	−0.00082	−0.00110	−0.00135	Logarithm of resistance in 1000's of ohms

* *For exact definition of moisture limits see Table I, previous paper (5).*

With the Burton-Pitt an increase in humidity gives higher readings, the increase being greater with grain of high moisture content. Owing to the fact, previously cited, that this instrument yields extremely erratic results on all samples giving a deflection of over 50 scale divisions, the coefficient calculated for damp grain is subject to some uncertainty. The effect of humidity on the Limbrick is relatively high at low moisture contents and decreases in magnitude as the moisture in the wheat increases. As the resistance to be measured in this instrument is high, the humidity effect may be due to an increased tendency toward surface leakage as the humidity increases. Surface leakage also offers an explanation for the higher humidity coefficient at low moisture contents, *i.e.*, higher resistances. The motor-operated Tag-Heppenstall has the smallest humidity coefficient of any of the three meters studied. The observed effect of humidity on this instrument can hardly be attributed to surface leakage as in the Limbrick, since the resistance measured is much lower. Furthermore, the effect increases with the moisture content of the wheat, a trend for which no explanation can be offered.

6. Calibration and Accuracy of Meters

Further subsamples of the 300 hard red spring wheat samples already referred to (5), were employed to determine the calibration and accuracy of the various meters. The temperature coefficients, described in § 4, were used to correct the observed readings for the small temperature fluctuations occurring in the conditioned room in which the tests were made. A standard temperature of 72° F. was adopted, with the usual correction of +3° in the Limbrick, to allow for the heating of the grain in grinding. Correction for the small fluctuations in relative humidity, from the standard 40% which occurred, was unnecessary since the humidity coefficients are so small.

Briefly the method of dealing with the results consisted of making the temperature corrections, calculating the standard error of observation ("experimental error") from the difference between the duplicate meter readings, and then plotting the vacuum oven moisture content against either the meter scale reading or, in certain resistance instruments, against the logarithm of some convenient multiple of the observed resistance. It was found that at moisture contents higher than about 17.0%, the relationship deviated significantly from the linearity observed at lower moisture contents, and as the errors of prediction were also somewhat larger at the high moisture contents, a separate curve was employed to represent the results in this moisture range. Since other investigators (3, 4) have shown that naturally damp grain behaves differently in certain electrical moisture meters from grain artificially dampened shortly before the tests, these two classes of damp wheat were analyzed separately, in spite of the fact that the artificially dampened samples had been tempered about a month before testing.

All the calibration curves were fitted by the method of Least Squares. The observed standard error of prediction of vacuum oven moisture content was computed, for the mean of duplicate determinations, from the sum of

the squares of the deviations of the observed values from the fitted curve. The net error of prediction was obtained from the observed error by allowing for the sampling error and the experimental error of the vacuum oven method.

The standard error of the duplicates, or "experimental error", was obtained in terms of meter scale reading or logarithm of the observed resistance, these being converted into terms of moisture by means of the appropriate calibration equation. When this was parabolic, the average moisture difference corresponding to the given instrumental deviation was computed from the average value of the first derivative.

Davies Meter

Preliminary experiments with the Davies meter demonstrated that it would be necessary to use three different sensitivities in order to obtain satisfactory readings on a series of samples of different moisture content. The only method of adjusting the sensitivity on this relatively simple instrument was to change the voltage. Sensitivity 1 employed a voltage of 100 and was necessary to give readable deflections with grain below 16.0% moisture. Sensitivity 2 corresponded to full-scale (100) deflection on the galvanometer with the standard resistance in the circuit, *i.e.*, a voltage of 46.86. Sensitivity 3 corresponded to a deflection of 30 scale divisions with the standard resistance in the circuit, a voltage of 14.0. Wherever possible the intermediate sensitivity was used, since this gave the closest approximation to the test conditions outlined by the manufacturers. Sensitivity 1 was used only with samples giving a scale deflection of less than 5, and Sensitivity 3 only with samples giving over full-scale deflections when the instrument was operated at intermediate sensitivity.

An examination of the results obtained showed that the meter was quite inaccurate and unsuitable for measuring the moisture content of wheat, the standard error of the duplicates ("experimental error") alone, for all samples tested at the intermediate sensitivity, being 5.9% of the average meter scale reading. Under these conditions it was not worthwhile converting the meter readings to resistance, fitting a curve to the points mathematically, or obtaining additional data on the effect of temperature. The results obtained are shown in Fig. 5 in which the vacuum oven moisture is plotted against the meter scale reading at the three sensitivities.

It is evident that even at the highest voltage (Sensitivity 1) the meter was not capable of distinguishing between "straight" and "tough" wheat, *i.e.*, below and above 14.5% moisture, since nearly all of the samples below 16.0% moisture gave deflections of less than 5 scale divisions. This alone renders it entirely unsuitable for commercial tests on wheat, even if the instrumental readings were reliable, and the large experimental error at Sensitivities 2 and 3 show that this is not so. The data plotted in Fig. 5 were not corrected for temperature variations, but as these never exceeded 3° or 4° F. such corrections would have little effect on the observed variability.

The large experimental error observed at the intermediate sensitivity suggests that the design of the test cell is faulty, for the scale deflections in this range were large enough to permit the meter to be read with reasonable accuracy. It is probable that the range of the instrument could be increased by the use of a larger test cell and a more sensitive galvanometer. In order

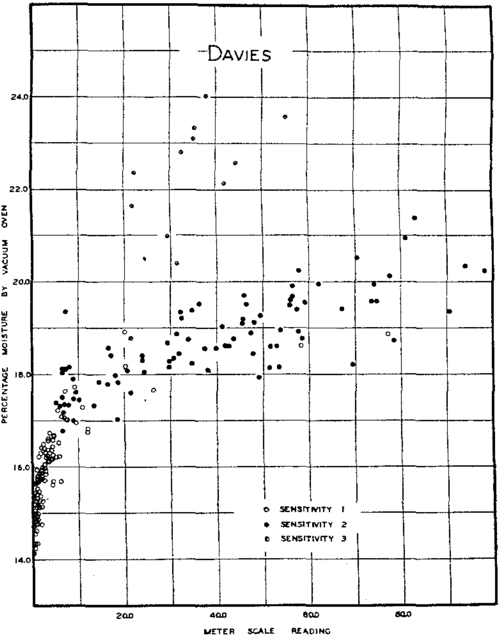

FIG. 5. *Relation between moisture content of wheat and Davies meter reading.*

to cover the wide range of moisture content experienced commercially, however, several sensitivities would still be required, together with a corresponding number of calibration charts. This is necessitated by the fact that an electrical measuring instrument such as a galvanometer, when used directly without a shunt or adjustable resistances, follows Ohms' law, *i.e.*, a rectangular hyperbolic relationship between deflection and resistance at constant voltage. On the other hand, the change of resistance of grain with increasing moisture

content is logarithmic (see Figs. 7, 8 and 9), and when a galvanometer is used directly to observe such resistances, the deflections at high resistance (low moisture content) will all be too small to permit distinction between two samples of slightly different moisture content, while at high moisture contents (low resistance) the meter will show enormously increased deflections for negligible differences in moisture content. An additional disadvantage arises from the fact that at high voltages there is a greater possibility of errors due to polarization.

Burton-Pitt Tester

As the result of several preliminary experiments, a sensitivity of 90 scale divisions, when the constant capacity condenser was in the circuit, was selected for the main series of experiments with the Burton-Pitt tester. At this sensitivity, five scale divisions corresponded to approximately 1.0% change in moisture content, and it was felt that had a lower sensitivity been employed, the errors of reading the milliammeter might have affected the accuracy of the results. A full-scale deflection of 100 divisions on the other hand would have limited the measurable moisture range to about 21.5%, and as few samples of higher moisture occur in commercial practice, it would have been difficult to establish a reliable supplementary calibration curve at a lower sensitivity.

The results obtained are shown in Fig. 6, and it is evident, as in the temperature and humidity studies, that the meter is decidedly erratic at deflections greater than 50 scale divisions, corresponding to moisture contents above 17%. The curve fitted to the points at lower moisture contents was parabolic, the equation being $M_V = 6.87 + 0.279x - 0.00152x^2$, where M_V is the moisture content by the vacuum oven, and x the Burton-Pitt reading in scale divisions at a sensitivity of 90. This slightly curved relationship between moisture content and scale reading was observed by Coleman (4), working with the D-K apparatus, which is also of the dielectric type, although Burton and Pitt (3) claim the existence of a linear relationship.

The standard errors of the mean of duplicate determinations made with this instrument ("experimental errors") were as follows: 0.06% moisture over the moisture range 11.0 to 17.0% covered by the curve; and 0.10% for all samples of higher moisture content. The experimental error at low moisture contents is therefore only slightly higher than those observed with the analytical methods, but at high moisture contents the meter is definitely less consistent. Apparently the circuit, or some part of the instrument, is too unstable to give constant results at scale deflections over 50. The observed standard error of prediction of vacuum oven moisture over the range covered by the curve was 0.55% moisture, and 0.54% after allowing for the proportion of the variance due to the sampling error and the experimental error of the vacuum oven determination. The error of prediction of moisture content is therefore much larger than the "experimental error" estimated from duplicate determinations on the same sample, a result similar to the findings with the analytical methods (5). The high error of prediction

shows that this meter is relatively inaccurate for determining the moisture content of wheat at moisture contents below 17.0%, and at higher moisture contents it is of no practical utility.

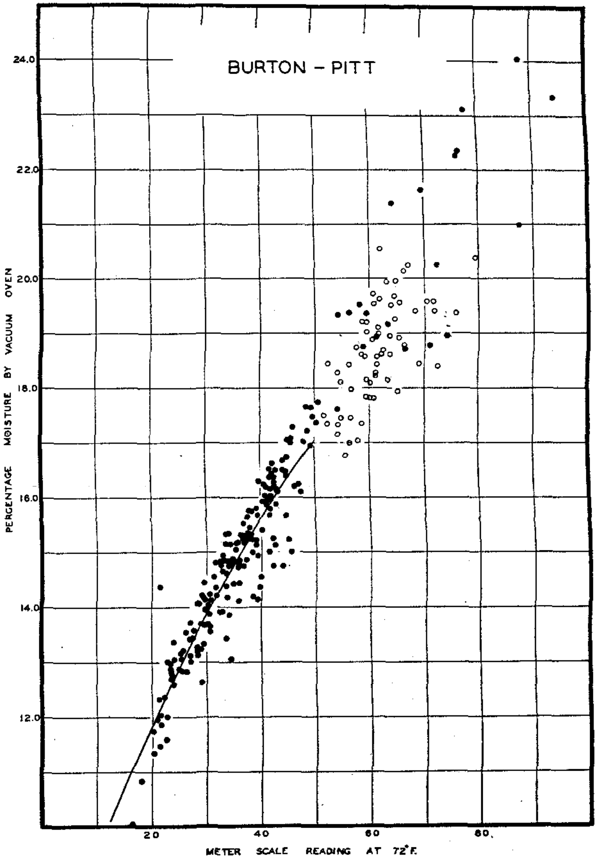

FIG. 6. *Relation between moisture content of wheat and Burton-Pitt meter reading. Hollow circles represent artificially dampened samples.*

Limbrick Tester

The Limbrick tester was used at its maximum sensitivity, *i.e.*, full-scale deflection on minimum resistance. Under these conditions its upper moisture limit was about 21.5% moisture, and its lower limit about 11.5% moisture. By altering the setting of the sensitivity the instrument can be made to measure grain of higher moisture content, but it is impossible to increase the sensitivity further to include drier grain.

The moisture content of the samples falling within the range of this meter is plotted against the logarithm of the resistance in megohms at 75° F. in Fig. 7. Four curves were fitted to the results, one representing the linear relationship at moisture contents below about 17.0%, another (broken line) the relationship with all samples of higher moisture content, and, as the artificially and naturally damp samples gave significantly different results, separate curves are shown for these.

It is evident that with grain of high moisture content the relationship between percentage moisture and resistance is parabolic, a given increment of moisture causing a progressively smaller change in resistance. Owing to the relatively small number of samples of high moisture content, the curves are determined with some uncertainty at the ends of the range, and do not meet exactly the curve obtained at lower moisture contents. Both the naturally and artificially damp samples show a similar curved relationship, the main difference being that the position of the curve is lower with artificially dampened samples. This point is discussed in greater detail towards the end of this section.

TABLE VI

Experimental error, calibration equations, and observed and net standard error of prediction of vacuum oven moisture content of wheat by the Limbrick moisture tester

Moisture range	Experimental error (standard error of duplicates), % moisture	Calibration equation*	Standard error of prediction	
			Observed moisture, %	Net moisture, %
11.5 to 17.0%	0.08	$M_V = 18.39 - 1.96 \log R$	0.40	0.38
17.0 to 21.5% (all samples)	0.09	$M_V = 19.43 - 4.65 \log R + 2.32 (\log R)^2$	0.51	0.49
17.0 to 21.5% (naturally damp)	0.07	$M_V = 19.76 - 4.92 \log R + 2.39 (\log R)^2$	0.48	0.46
17.0 to 21.0% (artificially damp)	0.10	$M_V = 19.36 - 4.39 \log R + 1.71 (\log R)^2$	0.46	0.44

Standard errors apply to mean of duplicates.

* M_V = *moisture content as determined by vacuum oven.*

R = *resistance in megohms.*

The experimental error, calibration equation, and observed and net error of prediction applicable to each series, are given in Table VI. The net error

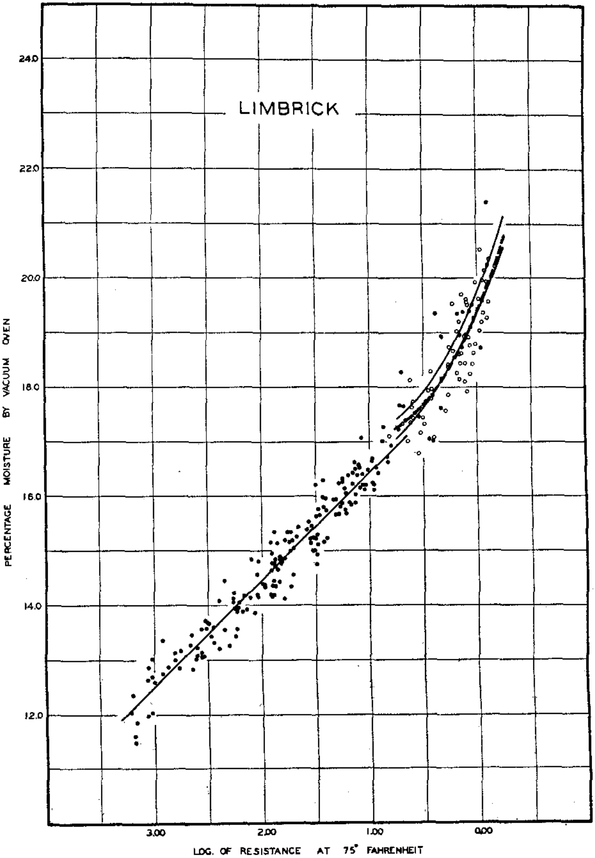

FIG. 7. *Relation between moisture content of wheat and logarithm of resistance in megohms. Limbrick meter. Hollow circles represent artificially dampened samples.*

strictly attributable to the instrument is estimated by allowing for the proportion of the residual variance due to sampling error and experimental error of the vacuum oven. The "experimental error" at low moisture contents is actually somewhat higher in terms of resistance than at high moisture content, a result which can doubtless be attributed to the difficulty of measuring the extremely high resistances accurately. The error of prediction is again much larger than the experimental error, and is somewhat larger for samples above 17.0% moisture. The slightly smaller error of prediction with the artificially dampened samples can doubtless be partially attributed to the fact that they fall in a lower moisture range than the naturally damp samples.

Hand-operated Tag-Heppenstall Meter

The Tag-Heppenstall instruments employ an Ayrton shunt, and are capable of covering a wide range of moisture content. Other than the use of the appropriate shunt stud there is no method provided for altering the sensitivity. The hand-operated instrument was operated at the roll-setting nearest to 40/1000 in., the spacing recommended in the manufacturer's instructions.

The results obtained are shown in Fig. 8, in which the vacuum oven moisture content is plotted against the logarithm of the resistance in thousands of ohms. They show the same general characteristics as those with the Limbrick, namely, a linear relationship at moisture contents up to about 17.5%, and then a parabolic relationship at higher moisture contents. As the artificial and naturally damp samples gave significantly different results, separate curves are shown for these. Again the curves do not meet exactly,

TABLE VII

Experimental error, calibration equations, and observed and net standard error of prediction of vacuum oven moisture content of wheat by the hand-operated Tag-Heppenstall moisture meter

Moisture range	Experimental error (standard error of duplicates), % moisture	Calibration equation*	Standard error of prediction	
			Observed moisture, %	Net moisture, %
11.0 to 17.5%	0.04	$M_V = 23.78 - 2.46 \log. R$	0.42	0.40
17.5 to 24.0% (all samples)	0.05	$M_V = 28.77 - 7.39 \log. R + 1.15 (\log. R)^2$	0.46	0.44
17.5 to 24.0% (naturally damp)	0.06	$M_V = 28.78 - 7.38 \log. R + 1.18 (\log. R)^2$	0.65	0.64
17.5 to 21.0% (artificially damp)	0.05	$M_V = 26.84 - 5.54 \log. R + 0.71 (\log. R)^2$	0.38	0.35

Standard errors apply to mean of duplicates.
* M_V = *moisture content as determined by vacuum oven.*
R = *resistance in 1000's of ohms.*

owing to the uncertainty which is inevitable at the end of the range with the small number of samples in the high moisture, naturally damp group. The errors and equations applicable to these curves are given in Table VII.

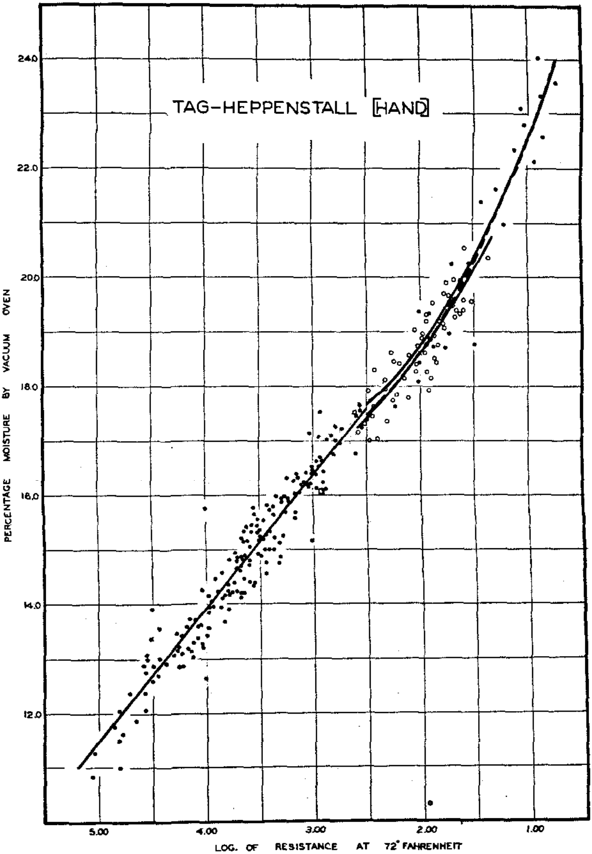

FIG. 8. *Relation between moisture content of wheat and logarithm of resistance in 1000's of ohms. Hand-operated Tag-Heppenstall meter. Hollow circles represent artificially dampened samples.*

The "experimental error", in terms of moisture, of this instrument is somewhat lower than that observed with the preceding meters. It seems probable that larger rollers, or a different roll spacing, would give even smaller experimental errors, for it was observed, during the course of the experiments, that the wheat did not feed into the rolls uniformly at the roll spacing used.

The net standard error of prediction, after allowing for the sampling error and the experimental error of the vacuum oven determination, is slightly higher than for the Limbrick instrument in the linear part of the moisture range. It is evident from Fig. 8 that a few of the points show a considerable divergence from the line, and these contribute considerably to the variance. Figs. 7, 8 and 9 also reveal that the upper moisture limit varied somewhat in the experiments with the different instruments. Thus 21.5% was the maximum moisture content that could be measured with the Limbrick instrument at the sensitivity used, while the Tag-Heppenstall instruments could be used for all samples, the highest moisture content encountered being about 24.0%. Since the meters all tend to become less accurate as the moisture content increases, the range of moisture contents included in this "damp" class must be considered in relation to the standard error of prediction for this range. In spite of the higher average moisture content of the samples tested in the damp range with the hand-operated Tag-Heppenstall meter, however, the error of prediction for all samples is lower than that obtained with the Limbrick instrument. The lower error of prediction with artificially damp samples as compared with the naturally damp group may again be partially explained by their lower average moisture content.

Motor-operated Tag-Heppenstall Meter

The roll spacing of the motor-operated Tag-Heppenstall was adjusted to 40/1000 in., as required by the instructions, by means of the shims provided. The calibration curves, shown in Fig. 9, are again of essentially the same type as those obtained with the hand-operated model and the Limbrick. There was again a significant difference between the results with artificial and naturally dampened samples. The calibration equations, and the errors applicable to each, are given in Table VIII. The experimental errors were lower with this instrument than with any of the other meters or analytical methods studied. Within the linear moisture-resistance range, this meter is much the most accurate of those tested, the net standard error of prediction being 0.16% moisture. The error of prediction for all samples of higher moisture content was larger than for either the Limbrick or hand-operated Tag-Heppenstall. It should be mentioned, however, that as the upper moisture limit of the Limbrick is lower than that of the Tag-Heppenstall meters a number of the wettest samples studied could not be tested in the Limbrick. As the accuracy of all the meters decreased with increasing moisture content this may account, in part, for the larger errors observed in the motor Tag-Heppenstall as compared with the Limbrick meter. There is no evident reason however for the motor-model being less accurate in this

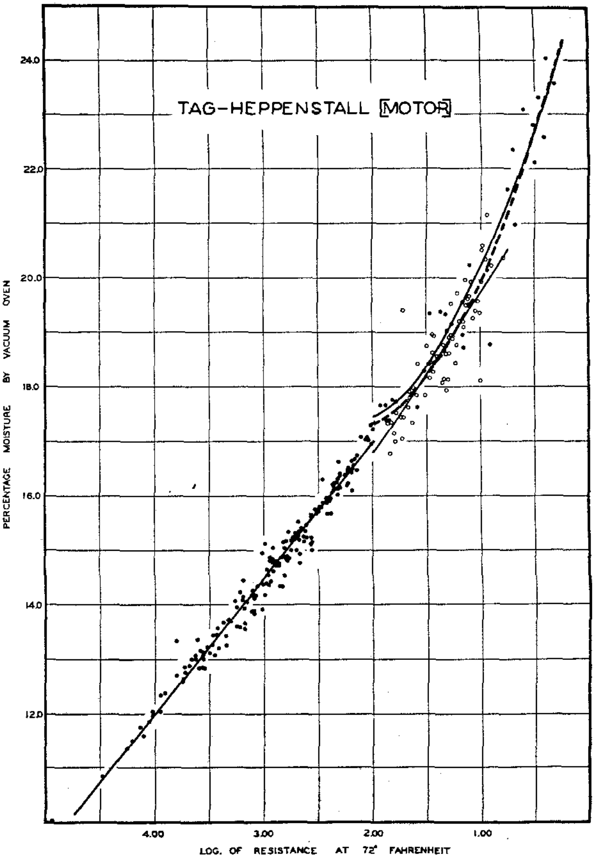

FIG. 9. *Relation between moisture content of wheat and logarithm of resistance in 1000's of ohms. Motor-operated Tag-Heppenstall meter. Hollow circles represent artificially dampened samples.*

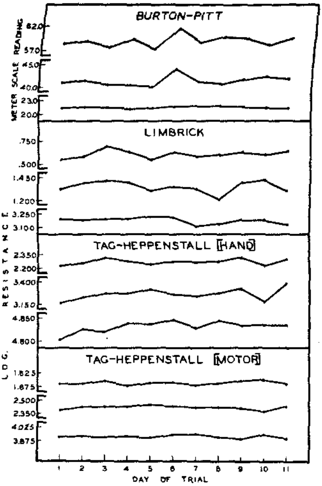

FIG. 10. *Daily fluctuations in meter results.*

range than the hand-operated type, except that two of the points, as shown in Fig. 9, exhibit considerably more departure from the curves than any of the others. The lower error of prediction with artificially damp samples may again be explained by the lower average moisture content of the former.

Day-to-day Variations

Subsamples of three large samples, one from each of the straight, tough, and damp, moisture classes, were tested daily in all meters in order to determine the extent to which the results were affected by daily fluctuations. The observed meter reading on these three samples is shown plotted against the day of test, for all meters except the Davies, in Fig. 10. Where the curves rise or fall together on a particular day, this is an indication of

TABLE VIII

Experimental error, calibration equations, and observed and net standard error of prediction of vacuum oven moisture content of wheat by the motor-operated Tag-Heppenstall moisture meter

Moisture range	Experimental error (standard error of duplicates), % moisture	Calibration equation*	Standard error of prediction	
			Observed moisture, %	Net moisture, %
11.0 to 17.0%	0.02	$M_V = 21.93 - 2.48 \log. R$	0.21	0.16
17.0 to 24.0% (all samples)	0.03	$M_V = 26.21 - 8.08 \log. R + 1.82 (\log. R)^2$	0.58	0.56
17.0 to 24.0% (naturally damp)	0.03	$M_V = 26.07 - 7.48 \log. R + 1.58 (\log. R)^2$	0.68	0.67
17.0 to 21.0% (artificially damp)	0.03	$M_V = 23.01 - 3.44 \log. R + 0.17 (\log. R)^2$	0.46	0.43

Standard errors apply to mean of duplicates.
* M_V = *moisture content as determined by vacuum oven.*
R = *resistance in 1000's of ohms.*

TABLE IX

EFFECT OF DAILY FLUCTUATIONS IN THE VACUUM OVEN AND METER RESULTS

Meter	Standard error of prediction of vacuum oven moisture in range 11.0 to 17.0%	
	Calculated around general mean, % moisture	Calculated around daily means, % moisture
Burton-Pitt	0.54	0.50
Limbrick	0.38	0.36
Tag-Heppenstall (hand)	0.40	0.38
Tag-Heppenstall (motor)	0.16	0.14

a daily fluctuation, while divergent results can be attributed to experimental or sampling errors. The curves suggest the existence of slight daily fluctuations with all meters except the motor-operated Tag-Heppenstall. (Note Burton-Pitt results sixth day; Limbrick results fifth day; and hand-operated Tag-Heppenstall results first day). Nevertheless, an analysis of variance showed that these systematic daily fluctuations could not be judged significantly greater than the erratic fluctuations, on the number of observations available.

It has already been shown (5) that there are significant fluctuations in the vacuum oven results from day to day. In obtaining the net error of prediction applicable to the meters, as given earlier, the experimental error of the vacuum oven and the sampling error only were allowed for. The effect of daily fluctuations in both the vacuum oven and the meter under test can, however, be eliminated by calculating the variance and covariance around the daily means, instead of around the means for the entire series. When this is done the standard error of prediction for the linear part of the range below about 17.0% moisture was reduced as shown in Table IX. Although the reduction in each case is statistically significant, the effect from a practical point of view is negligible. Whether this reduction in the standard error can be attributed entirely to the daily variations in the vacuum oven, or whether it is also due in part to daily fluctuations in the meters, cannot be said with assurance, but it seems likely that part of the reduction in the error of the Burton-Pitt meter at least, is due to the presence of daily fluctuations in the results by that instrument.

EFFECT OF ARTIFICIAL DAMPENING

Since the artificially dampened wheat had been allowed to stand about a month between tempering and the performance of the tests, it was felt that these samples should behave as naturally damp in the electrical testers. Nevertheless, since Coleman (4) had shown that artificially dampened samples do not give the same results as naturally damp samples, at least for several days after tempering, the results from the two classes of wheat were examined separately. It was found that in the three most accurate meters there was a significant difference, the artificially dampened samples exhibiting on the

average a slightly higher conductivity than naturally damp samples of the same apparent moisture content. As, however, the natural and artificially dampened samples were not tested on the same days, the situation may be complicated by the effects of daily fluctuations. The presence of small but significant fluctuations affecting all the results obtained on a particular day has already been demonstrated in the straight and tough samples (Table IX) when tested by any of the foregoing meters. A more detailed examination of the results with these straight and tough samples revealed that those tested on the same days as the artificially dampened also showed a slightly higher conductivity, when reduced to a common moisture basis, than those tested on the days when naturally damp samples were employed. As this differentiation is common to all three meters, daily fluctuation in the vacuum oven results suggests itself as the underlying cause. It thus seems likely that part, if not all, of the observed difference between the naturally damp and artificially dampened samples may be attributable to similar daily fluctuations.

7. Effect of Grade and Variety of Wheat

Burton and Pitt (3) state that they are confident that the relationship which they obtained between moisture content and current strength (scale reading) holds for all ordinary grades and varieties of wheat, but also state that for accurate work it will be necessary to calibrate the apparatus for the particular variety tested. In addition to the possibility of a different calibration curve being required for different grades of wheat, it was felt that the presence of a number of lower grade samples might add to the variability, since grain may be of low grade for many different reasons.

In order to study the effect of grade, the 37 samples of grade No. 4 Northern or lower, which occurred within the linear range below 17.0% where the meters are most accurate, were compared with 37 samples grading No. 1 Hard, No. 1 or No. 2 Northern. The latter were selected to have about the same moisture content as the low grade samples, and the same number of high and low grades were selected from each day of test so as to equalize any effect of daily fluctuations. The data, plotted in Fig. 11, show no evidence of the need of a separate calibration chart for the high and low grades nor do they indicate that the low grade were significantly more variable than the high grade samples. These conclusions were confirmed by an analysis of variance. To obtain this result with the Tag-Heppenstall instruments is rather surprising, since the less uniformly sized kernels common to the lower grades might reasonably be expected to give different results when the same roll spacing is used throughout. However, the crop year 1933-34 provided relatively few types of low grade wheat such as frozen, rusted, sprouted or artificially dried, which occur in certain seasons. It should be mentioned, moreover, that all of the samples were carefully cleaned before testing, and the presence of dirt, weed seeds or other impurities may affect the accuracy of the instruments in commercial practice.

study of varieties was limited to a comparison of Garnet with the 1 spring wheat varieties represented in the 300 samples employed in eral reliability test, owing to the difficulty of obtaining pure varieties a wide range of moisture content. This variety, since it has a distinctly

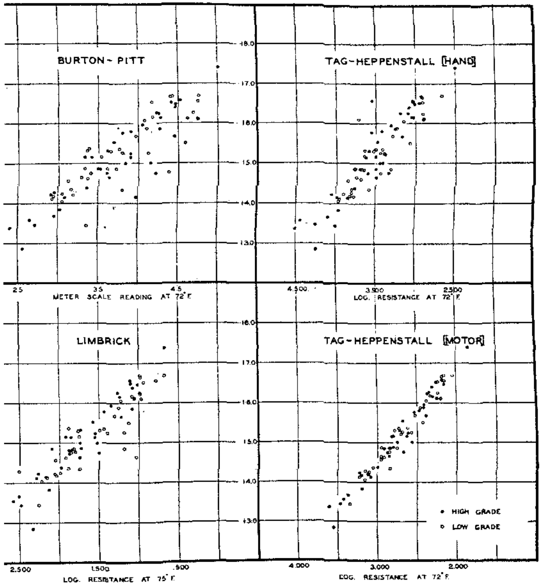

FIG. 11. *Comparison of meter results with high- and low-grade wheats.*

kernel than the other spring wheats tested, should show whether any ce is likely to occur in the behavior of varieties in the electrical e meters.

28 Garnet samples used all fell within the straight and tough moisture The results obtained with the four moisture meters are plotted in together with the calibration curves already obtained from the general ty test. It is evident that this curve fits the points for all instruments, the usual variation, except the hand-operated Tag-Heppenstall. With trument the resistance, at a given moisture content, appears to be higher for Garnet than for the other samples. It has already been men-

tioned that in the earlier tests the wheat did not feed into the rollers of this instrument in an entirely uniform manner at the roll spacing recommended. This effect was even more pronounced with the harder kernelled Garnet, and the number of kernels caught between the rolls at any instant was probably smaller than with the softer wheats, thus accounting perhaps for the slightly higher resistance observed. Otherwise there is no evidence to indicate that a different calibration curve is required for different spring wheat varieties.

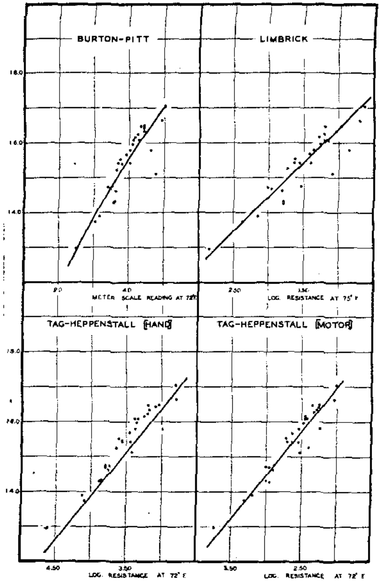

FIG. 12. *Comparison of meter results with Garnet and other hard red spring wheat varieties.*

Although no extensive study of the meter adjustments was contemplated, it seemed advisable to give the roll spacing in the hand-operated Tag-Heppenstall meter some consideration, since it was observed that the fluctuations of the microammeter needle were much greater with Garnet than with the other hard red spring wheat varieties, a finding which might be attributed to the uneven manner in which this wheat fed through the rolls. The spacing is adjusted by means of an eccentric arm with settings numbered from 1 to 5, the distance between the rolls increasing with the numbers. Setting No. 3, found to be $40/1000 \pm 1/1000$ in. was used in all previous experiments, in accordance with the manufacturer's recommendations. In a supplementary experiment, settings 4 and 5 were also used, though the spacings these provide are not known. The standard error of the mean of duplicates with the Garnet samples at the above settings was found to be: No. 3, 0.12% moisture; No. 4, 0.08% moisture; and No. 5, 0.06% moisture. It is evident that the wider spacings give better results with this variety, and it is probable that a somewhat similar result would be obtained with other spring wheat varieties. The roll spacing employed, and meter adjustments generally, should be subjected to critical study, as the accuracy of some of the meters might thus be improved.

8. Tests with Wheat of Low Moisture

Although the calibration curves given earlier cover the moisture range of main commercial importance, a few experiments were performed with very dry wheat in order to determine the lowest moisture content the meters were capable of measuring, and also to ascertain whether the calibration curves already determined could be extrapolated to include such low moisture contents. Only the Burton-Pitt and the two Tag-Heppenstall meters were employed in this experiment, the lower limit of the Davies and Limbrick being already attained in the calibration experiments.

The results obtained are shown in Fig. 13, the points indicating the observed values, while the lines are an extrapolation of the calibration curve previously given, obtained by substituting the observed resistances in the

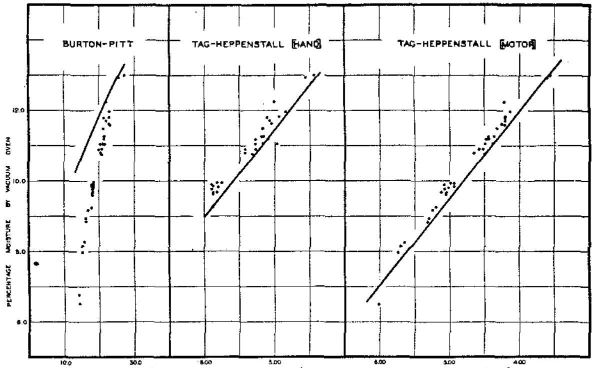

FIG. 13. *Meter results with wheat of low moisture content.*

calibration equations. It was found that the hand-operated Tag-Heppenstall was not capable of determining moisture contents lower than about 9.0%, but the motor-operated model could be read at moisture contents as low as 7.0%, since the roller electrodes are much larger, and the observed resistances proportionately smaller.

The Burton-Pitt gave appreciable deflections even on the driest wheat employed, but it is evident from Fig. 13 that the points do not follow the curve deduced from wheat of higher moisture contents, a given decrement of moisture causing progressively smaller change in the meter reading. This decreasing sensitivity makes it probable that the error of prediction at moisture contents below about 10% would be relatively large.

The results with the two Tag-Heppenstall instruments follow closely the calibration curve obtained at higher moisture levels, but the points are in

general above the line. It is possible that this may represent a real difference *e.g.*, these dry wheats may not feed into the rollers as uniformly as the softer kernels of moister wheat, but it may also be explained by daily variations in the vacuum oven, as previously noted, since all these samples were tested on one day.

9. Corrections for Variations in Temperature and Humidity

The temperature coefficients in terms of meter reading and logarithm of resistance have already been given in Tables III and IV. In commercial practice, it is more convenient (though less accurate if the calibration is non-linear) to present these temperature coefficients in terms of correction values, showing the amount of moisture to be added or subtracted for each degree F. the temperature is below or above that at which the calibration curve or table is applicable. Since the temperature coefficient and the relation between meter reading, or resistance, and the moisture content both vary with the moisture content, it is necessary to give the correction applicable to different moisture ranges separately. The average temperature coefficients for straight, tough and damp grain respectively were therefore computed from the data presented earlier in Table IV, and are summarized in Table X.

It is evident that the temperature correction applicable to the wheat-instrument system increases with increasing moisture content in the Burton-Pitt and hand-operated Tag-Heppenstall, while it tends to decrease with moisture content in the Limbrick, and is remarkably constant throughout the entire moisture range with the motor Tag-Heppenstall meter. The temperature correction applicable to the wheat only, is, for reasons already given, somewhat less precisely determined. Nevertheless, there is no doubt that these two corrections are different in both the Burton-Pitt and Limbrick instruments, where the correction for the wheat only is little more than half that applicable to the wheat and the instrument. There is little difference between the two corrections for the motor Tag-Heppenstall instrument, indicating that the meter itself is little affected by temperature. This finding doubtless applies to the hand-operated model also, since the meter unit is the same.

The question arises as to the temperature range over which these corrections may be applied. The corrections for the wheat–instrument system were determined over the range 60° to 90° F., but since they are linear for the better resistance type meters, they may with reasonable assurance be applied over any range likely to be encountered in practice. The standard error of these corrections for the resistance instruments in the straight and tough moisture ranges is usually not greater than about $\pm 0.001\%$ moisture per °F. (see Table IV), so that they are accurate enough to be applied over a $\pm 50°$ F. range without introducing an error greater than 0.05% moisture. For the Burton-Pitt, the standard error of the corrections is such that the recorded values can only be used over a range of about $\pm 10°$ F. if the same accuracy is required. The corrections for variable wheat temperature only, in the Burton-Pitt and Limbrick instruments, should not be applied over a

greater range than about ±10° F. owing to their uncertainty. With the Tag-Heppenstall instruments the two temperature corrections are so nearly the same that the correction for the wheat–instrument system could be applied to the wheat alone over a range of ±10° F. without introducing significant error. The superiority of the Tag-Heppenstall instrument over the others in this regard is evident.

When an accurate result is required in commercial practice, the grain should be allowed to stand in the test room until it has attained approximately the temperature of the instrument, before the determination is made. The temperature of the wheat should be taken, and as it will then be within a few degrees of the temperature of the room and instrument, the temperature correction for the wheat–instrument system can be used to correct the results without significant error. No attempt should be made, in accurate work, to apply the temperature corrections for the grain alone over a range of more than 10° F., for not only are they themselves uncertain for larger temperature ranges, but difficulty will be experienced in determining the temperature of the grain accurately. It is evident from Table X that an error of 1° F. corresponds to about 0.05% moisture, and it is difficult to measure the temperature of grain which differs from the surrounding atmosphere by more than 10° F. with this degree of accuracy.

The corrections for humidity variations were computed from the humidity coefficients in the manner previously described, and are also given in Table X.

TABLE X

TEMPERATURE AND HUMIDITY CORRECTIONS IN TERMS OF PER CENT MOISTURE

Meter	Standard temperature, °F.	Correction in terms of per cent moisture for each degree F. above or below the standard temperature					
		Straight		Tough		Damp	
		Wheat and meter	Wheat only	Wheat and meter	Wheat only	Wheat and meter	Wheat only
Burton-Pitt	72	0.071	0.041	0.081	0.047	—	—
Limbrick	75	0.069	0.031	0.067	0.040	0.052	0.037
Tag-Heppenstall (hand)	72	0.054	—	0.057	—	0.066	—
Tag-Heppenstall (motor)	72	0.067	0.060	0.068	0.060	0.068	0.068
	Standard humidity at 72° F., %	Correction in terms of per cent moisture for each 10% change in relative humidity above or below standard humidity					
Burton-Pitt	40	0.017	—	0.021	—	—	—
Limbrick	40	0.070	—	0.065	—	0.060	—
Tag-Heppenstall (motor)	40	0.020	—	0.027	—	0.046	—

NOTE: *Corrections are to be* added *if the temperature or humidity are below the standard, and* subtracted *if above the standard.*

As variations in the humidity have a much smaller effect on the results, the corrections given apply to 10% rather than 1% changes in relative humidity.

The humidity correction is least for the Burton-Pitt and greatest for the Limbrick, and there is a decided tendency for the correction to vary with the moisture content of the wheat. The significance of this has already been discussed.

Since a 10% change in the relative humidity of the test room has about the same, or less, effect than a 1° F. change in temperature, corrections are unnecessary if the relative humidity is within ±10% of that at which the calibration curve is applicable. The values given, however, may be useful for correcting calibration tables, etc., to the average relative humidity found in different localities, and if this is done it is doubtful if humidity corrections need to be applied at the time of making the test unless the humidity is extremely variable. The corrections given in Table X may be applied with assurance over the range 20% to 70% relative humidity at 72° F.

10. Summary of Conclusions by Sections

Design and Operation of Instruments (Section 2)

Only one of the meters tested, the Burton-Pitt, was of the dielectric type. This was found to be inferior to all the resistance type instruments studied, with the exception of the Davies. It is less stable, requires more adjustment, and is more affected by the temperature of the test room.

The Davies meter is not suitable for determining the moisture content of cereals. It is not sufficiently sensitive to give appreciable readings with grain of less than 16.0% moisture, and the galvanometer employed to measure the transmitted current is not capable of following the logarithmic resistance-moisture relationship over the range of moisture encountered. This necessitates frequent changes of voltage and the use of several calibration curves. The irregularity of the results and the large experimental error suggest that the design of the test cell is also faulty.

The Limbrick and Tag-Heppenstall instruments seem to be reasonably satisfactory in design, though the higher resistances measured in the Limbrick tend towards high humidity coefficients, and the metallized resistors are not of as stable a quality as the wire-wound type which can be used when the resistance to be measured is smaller. The large roller electrodes of the motor-operated Tag-Heppenstall meter proved to be superior to the smaller ones of the hand-operated model, feeding more uniformly and giving more consistent results, but both possess advantages over the static test cell.

Samples to be tested must be weighed for the Burton-Pitt, ground and measured to constant volume for the Limbrick, and packed in the test cell of the Davies. No preliminary preparation of sample is necessary for the Tag-Heppenstall meters. The Limbrick was found to be the slowest and the Tag-Heppenstalls the most rapid in operation, duplicate tests with the motor model of the latter requiring on the average only 1.2 minutes.

Neither the Burton-Pitt nor the motorized Tag-Heppenstall can be considered unconditionally portable instruments, for in addition to their bulk and weight, they require electrical power for operation.

Effect of Temperature and Humidity (Sections 4 and 5)

Results by all the meters studied were appreciably affected by temperature conditions, and also to some extent by the humidity. An increase in temperature increases the conductivity of the wheat. The temperature coefficient of the wheat alone was found to be appreciably different from the combined temperature coefficient of the wheat and Burton-Pitt or Limbrick tester. The temperature coefficient of the wheat–instrument system in terms of resistance or meter reading decreases with the moisture content of the grain in all the meters studied, but owing to the form of the calibration curve, when converted to a moisture basis it increases with moisture in the Burton-Pitt, decreases in the Limbrick, and is practically constant in the motor Tag-Heppenstall.

The effect of humidity also varies somewhat with the moisture content of the grain. It is most pronounced in the Limbrick, but in most cases a 10% change in relative humidity at 72° F. has less influence than a 1° F. change in temperature.

Calibration and Accuracy of Meters (Section 6)

A slightly curvilinear relation exists between the Burton-Pitt reading and the moisture content of wheat over the range 11.0 to 17.0%; at higher moisture contents this instrument gives erratic results. All the resistance type meters show a linear relation between moisture content and the logarithm of the resistance over the range 11.0 to 17.0% moisture. Above this point given increments of moisture cause a diminishing change in resistance, the relationship becomes parabolic, and the estimation of moisture progressively less precise, as shown by the experimental errors and the net standard errors of prediction given in Table XI. In all cases the "experimental error", as judged from the deviation of duplicate readings, represents only a fraction of the error of estimation of moisture content.

TABLE XI

Experimental error and net standard error of prediction of vacuum oven percentage moisture by electrical moisture meters

Meter	Experimental error (standard error of duplicates)		Net standard error of prediction	
	Moisture range 11.0 to 17%	Moisture range over 17%	Moisture range 11.0 to 17%	Moisture range over 17%
Burton-Pitt	0.06	0.10	0.54	—
Limbrick	0.08	0.09	0.38	0.49
Tag-Heppenstall (hand)	0.04	0.05	0.40	0.44
Tag-Heppenstall (motor)	0.02	0.03	0.16	0.56

Standard errors apply to mean of duplicates.

It is evident from Table XI that the meters fall in the following order of decreasing accuracy over the moisture range 11.0 to 17.0%, which is most important commercially: motor-operated Tag-Heppenstall, Limbrick, hand-operated Tag-Heppenstall and Burton-Pitt, the Davies being of no practical utility. Above 17.0% the Burton-Pitt gave quite erratic results. From a practical standpoint the Burton-Pitt is definitely inferior to the others, the Limbrick and the hand-operated Tag-Heppenstall about equal, and the motor-operated Tag-Heppenstall definitely the best over the lower, more important, moisture range, and only slightly, if at all, inferior to the Limbrick at higher moisture contents.

The moisture range of the different meters is not identical. The Burton-Pitt is capable of a wide range provided the sensitivity is changed. The Limbrick cannot be used to measure moisture contents lower than 11.5%, while 21.5% is the upper limit when set at the maximum sensitivity. This instrument can be adjusted to read grain of higher moisture content, but an additional calibration curve is then required. The lower limit of the hand-operated Tag-Heppenstall is about 9.0%, and that of the motor model about 7.0%. The upper limit of both is probably between 25 and 30%, but as with all the meters this is really determined by the increasing uncertainty of the result.

EFFECT OF GRADE AND VARIETY OF WHEAT (SECTION 7)

Comparison of the limited number of low grade samples available with those of higher grade indicated that both high and low grade samples followed the same calibration curve in any particular meter, and there was no evidence of greater variability in the results obtained with the lower grades. Tests with Garnet indicated that in general it followed the same calibration curve as the other hard red spring wheat varieties represented in the general reliability series, in spite of the fact that the kernels of Garnet are harder. In the hand-operated Tag-Heppenstall meter however it showed a slightly higher resistance than expected. This seems to be due to inadequate roll spacing, and the accuracy of this instrument in general might be improved by using a wider spacing.

Acknowledgments

In addition to the individuals mentioned in the first paper of this series, the authors wish to acknowledge the assistance of Mr. D. S. Binnington and Mr. L. Sair, Research Assistants, Associate Committee on Grain Research, National Research Council of Canada, who were responsible for all of the meter determinations; special thanks are due to the former, who took charge of certain phases of the experimental work. They are also indebted to Mr. A. J. Grant, Physicist, National Research Laboratories, who measured the resistances and examined all the instruments before and after the investigation. Dr. J. T. Henderson, Physicist, National Research Laboratories, determined the temperature coefficient of several types of resistors.

The authors desire also to express their appreciation of the cordial co-operation of the individuals and firms who supplied the moisture meters for these tests, namely: the Davies meter, A. Gallenkamp and Co. Ltd., London; the Limbrick tester, Keyes Supply Co. Ltd., Ottawa; the Tag-Heppenstall hand- and motor-operated meters, C. J. Tagliabue Mfg. Co., Brooklyn, N.Y. The Burton-Pitt meter used was the property of the National Research Council of Canada, but Professor Burton kindly contributed suggestions with regard to its operation.

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ON A METHOD FOR MEASURING THE RESISTANCE OF VEGETAL TISSUES[1]

By Henri Prat[2]

Abstract

A method is described whereby numerical evaluations are introduced in the study of properties which in the past have been expressed qualitatively, namely, mechanical resistance and stages of maturation of vegetal tissues.

The building and operation of the apparatus are described. The results obtained in the case of growing and adult cereal straws are given. These reveal some important features in the mechanical construction of the culms of grasses.

Introduction

In the field of experimental plant morphology many data are qualitative rather than quantitative. For instance, stages of maturation of growing tissues, consistency of different tissues of a stem, etc., are usually described by force of circumstance in the general terms of "more" or "less" resistant without the possibility of giving numerical values to these terms.

Furthermore, in comparing different specimens, the difficulty is still greater. With these considerations in mind, this work was undertaken, in the hope of developing a method which would give more precise information in units that would allow of inter-comparisons being made between the various observations, at least within the limits of one set of experiments.

Apparatus

The first apparatus employed consisted of plain Roberval scales transformed by the addition of a cutting blade and a platform moved by a system of levers. Measurements of cutting strength obtained by adding weights to the apparatus proved unsatisfactory, the method being slow and imperfect, and it was soon realized that this quantity could be quickly and accurately read only from a direct-reading scale, using either a spring (dynamometer) or a gravity system.

The pressure applied to the blade used to cut the stem must be easily controllable and must be applied in such a way that there are no sudden irregular increases in the pressure. The apparatus shown in Fig. 1 proved satisfactory. The blade is moved by means of the rack and pinion of a microscope, M, a rod holding a frame for the interchangeable blade, B, being attached in place of the objective.

The cutting blade may be that of any scalpel or razor, but ordinary safety razor blades are most satisfactory, for such blades, when worn out, can be easily replaced by strictly equivalent ones. Dr. A. Navez has made careful observations on commercial safety razor blades and has shown that their sharpness, mechanically obtained, is adequately standardized and constant.

[1] *Manuscript received May 15, 1934.*
Contribution from the Department of Biology, University of Montreal, Montreal, Canada.
[2] *Director of the Department of Biology, University of Montreal.*

Fig. 1 shows the general features of the apparatus equipped with a spring scale, as constructed in our laboratory at the University of Montreal. Fig. 2 shows the same apparatus fitted with a gravity scale. Such scales are preferable to spring scales, as their indications are more regular and progressive during application of the pressure.

The piece of tissue to be studied, for example a cereal culm, is fixed by some kind of strip to a narrow piece of wood, W, placed on the scale platform. It

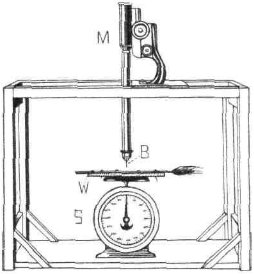

FIG. 1. *Outline of the apparatus equipped with a spring scale, S. M is the body of a microscope, B a movable blade, W a piece of wood supporting the stem.*

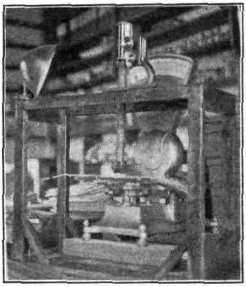

FIG. 2. *The apparatus equipped with a gravity-operated scale.*

is advisable to interpose between the stem and the wood several ribbons of bristol paper of appropriate width, to prevent the blade from touching the support before complete section of the tissue.

The moment of complete section of the culm can be detected by means of an electrical contact controlling a light or sound signal, but in general this improvement is not essential and the slight noise, shock, and jump of the piece is sufficient indication of completion of cutting.

The writer's experiments being performed with the intention of obtaining numerical data, as part of a general study of the growth of stems, and not for themselves alone, it was only after the completion of the experiments that the extent of the work of others along similar lines was realized. In this connection one may cite Albrecht (1), Holdefleiss (4) and Salmon (7). The property tested by these workers was *breaking strength*, measured by the application of pressure at right angles to the axis of a straw set up as a bridge between two supports. The quantity measured is thus practically "resistance to lodging."

The apparatus described in this present paper, devised to measure cutting and crushing strengths is however easily adaptable for the measurement of breaking strength. It is only necessary to place upon the platform a pair of supports upon which the straw rests (Fig. 4), and then to exert pressure

on the straw with the cylinder used in the determination of crushing strength. The writer intends to compare the breaking, crushing and cutting strengths of straws in a further publication.

Methods

Most of the stems examined were hollow cereal straws. If the hollow culm is placed entire on the testing platform, the measurement is often complicated by a preliminary crushing effect which occurs before cutting starts. For example, when the middle of an internode is sectioned, the following succession of phases is observed:—

(i) As progressively increasing pressure is applied, the stem is at first elastically compressed only, without permanent deformation (Fig. 3, A).

(ii) When the pressure reaches a certain value, the stem is crushed, and breaks into many narrow, distinct strips (Fig. 3, B).

(iii) After this crushing, the blade is working on a flat surface and for a time, as the pressure increases, the tissues are again in a state of elastic compression prior to actual penetration of the blade.

(iv) Finally the blade begins to section the peripheral sheath of sclerenchyma, then the vascular bundles and other tissues, and finally complete section is accomplished under a pressure easily read on the scale.

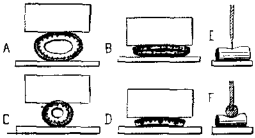

FIG. 3. *The cutting of a straw: A, elastic compression; B, crushing; C, sectioning of the solid portion of a straw, where cutting begins during the phase of elastic compression; D, cutting a split culm; E, measurement of cutting strength, with a blade; F, measurement of crushing strength, with a cylinder.*

It is necessary then, to consider two entirely distinct values, the crushing pressure and the cutting pressure. It is interesting to separate these two quantities as far as possible.

To this end, it should be noted that the cutting strength depends not only on the qualities of the straw but also on the sharpness of the blade used, while the crushing strength, for a linear contact, is almost independent of the characteristics of the blade, and may be considered as a definite property of each point of the straw.

With these facts in mind two complete series of measurements are performed on each sample; one with a razor blade (Fig. 3, E), the other with a metallic cylinder in place of the razor (Fig. 3, F).

When plotted, the data thus obtained produce two entirely different curves. In the middle of a hollow internode, the cutting strength is greater than the crushing strength. At the extremities, the central lacuna being locally reduced (Fig. 3, C), the crushing strength increases rapidly, while the cutting strength on the other hand decreases (Fig. 8). Close to the nodes, if a sufficiently sharp razor is used, section begins during the phase of elastic compression, and is completed before any permanent crushing occurs. When cutting is attempted in the middle of an internode, the unavoidable pre-

liminary crushing may introduce an error into the measurement of the cutting strength, in that, by reason of the shock incidental to crushing, actual cutting may be initiated at a lower pressure than would otherwise have been necessary.

It seems advisable to eliminate this effect by operating on half culms, obtained by longitudinal splitting (Fig. 3, D). The action of the blade is then very regular, reaching at the same time all parts of the material offered on a flat surface, instead of successively cutting parts of a round body as in case C, Fig. 3, or two identical sets of tissues placed one upon another as in B, Fig. 3. This procedure has the advantage also of allowing operation on strips of tissue of exactly known width.

To enable intercomparisons to be made between cutting strengths, experiments must be made with strips of equal width, *i.e.*, strips making contact with an equal length of the edge of the blade. If this precaution is not taken it is necessary to correct the readings in proportion to the widths of the strips used.

Measurements of width and thickness are made with a caliper provided with a vernier. Section should always be performed with the same face of the split straw facing the blade. This face should preferably be the outer one.

To increase the accuracy of measurement it is necessary to consider the rate of increase of the pressure. The cutting strength varies with different rates of application of pressure according to a complicated function of the sharpness of the blade and the qualities of the stem. It is necessary, therefore, to perform experiments under constant conditions if the readings are to be compared one with another. The time elapsing between the first contact and complete section of the stem must be as constant as possible, for example 20 sec., and during this time, pressure must be applied by a very regular and progressive motion of the focusing screw of the microscope.

Cutting strength may be defined as the maximum resistance offered to cutting by a standard blade moving under a steadily and slowly increasing pressure. It is measured in terms of the maximum pressure which can be applied to the blade without effecting complete section of the stem.

Under such conditions the fluctuations and values obtained may be less than 10 or 20%. This approximation is sufficiently accurate on account of the wide range of values obtained, *i.e.*, from 10 to 3000 gm.

The values given by such measurements cannot be taken as absolute numbers. They refer specifically to a given apparatus, with a given type of blade, and a given rate of application of the cutting force. With this reservation they are useful in giving numerical relations where formerly only qualitative data were obtainable.

Greater accuracy can be obtained by repeating measurements on neighboring points and closing each series of measurements by returning to the first point of observation. One thus eliminates errors due to any alteration in the characteristics of the blade which may occur during the progress of the work. Values obtained around the same level of the culm are averaged.

When observations must be made on neighboring points on the stems, it is especially useful to operate on split culms, otherwise crushing of the straw in the first experiment leaves the material in an altered condition for the next measurement. Before starting an experiment it is advisable to trace a metric scale on the internodes with India ink or glass-marking pencil. These guiding marks permit more rapid operation, as it is unnecessary to use a ruler to locate the points at which sections are to be made.

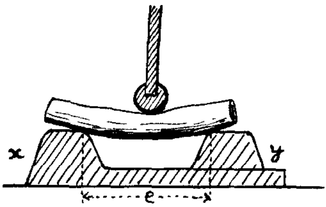

FIG. 4. *Adaptation of the apparatus for measuring breaking strength, x and y being supports which can be separated a variable distance, e, by means of a slide.*

The examples given below will show the application of the method.

Measurements

A. Young Culm

Consider first young culms with incompletely ripened tissues. Experiments may be performed under two conditions: (i) on the complete culm with the sheaths of the leaves wrapping the internodes, (ii) on bare internodes deprived of the leaves.

In the second case the resistance of a young culm is exceedingly low, particularly at the bases of the internodes. At these points the meristematic tissues of the growing zone are of an almost fluid consistency and the stiffness of the culm is due only to the rigidity of the sheathing leaves.

Fig. 5 shows resistances of a terminal internode of a flowering culm of a wheat (Blé Aurore), at the time of shedding of pollen (June 21, 1930). Measurements are relative to the bare internode and are taken on the whole straw without splitting.

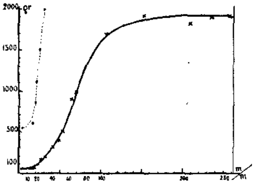

FIG. 5. *Resistance to section (cutting strength) of the terminal internode of wheat, at the time of anthesis (solid line). For clearness, the small values at the base of the internode are reproduced at the left (dotted curve), with the ordinates on a scale of 10:1 in comparison with the solid curve*

Taking a sigmoid shape, the curve rises from 55 gm. at the base, to a value of 1900 gm. at about the middle of the internode. This value is maintained throughout the upper part of the internode. In the same sample of wheat, resistance of the axis of the ear is about 2000 gm. at the base, increasing a little (to 3400 gm.), and then decreasing gradually toward the apex (to 1100 gm.).

B. Growing Culm

Consider now a younger culm not yet fully grown. Fig. 6 shows a growing culm of rye *(Secale cereale);* on the left is a schematic diagram of the culm,

and on the right are graphs of the resistance of the tissues at points corresponding to those marked on the diagram. Measurements were taken directly, without splitting the culm, and without correcting the readings in proportion to the diameters. However, the range of values is so great (from 20 gm. to 2800 gm.), that these corrections are not necessary to an understanding of the structure of the stem

Resistances of bare internodes are indicated by crosses and solid lines. It will be noticed that the inferior internode E_2 offers a high resistance throughout its length (from 1600 to 2800 gm.). Maturation of tissues is here complete.

The next internode E_3 is not entirely mature. In its upper part the resistance reaches 2500 gm., but at the base it is only 200 gm. This indicates the role played by the sheathing leaves in protecting the young stem. To show this still more clearly the resistances of the whole of each internode E with the sheathing leaf G, tested together, are indicated by circles joined by dotted lines (Fig. 6, G_3, G_4, etc.). It can be seen that $G_3 + E_3$ offers a resistance varying from 1100 to 1250 gm., instead of 200 gm. as offered by E_3 alone. The false node F_3 offers a low resistance (650 gm.), in full accord with its state of low differentiation. On the diagram on the left (Fig. 6) the parts of the internodes offering a resistance of less than 500 gm. are shown in black, those showing a resistance between 500 and 1000 gm., in cross-hatching, and those showing over 1000 gm., in white. Thus the superposition of very young meristems and consolidated tissues is clearly shown.

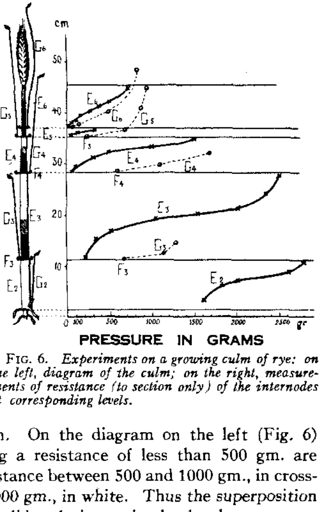

FIG. 6. *Experiments on a growing culm of rye: on the left, diagram of the culm; on the right, measurements of resistance (to section only) of the internodes at corresponding levels.*

Internode E_4 offers in general a low resistance (under 1000 gm.). The highest value is 1500 gm. and the lowest 70 gm.

Internode E_5 is still younger and weaker; its resistance is 45 gm. at the base and 150 gm. at the top. It is entirely meristematic, but in compensation it is supported not only by its own leaf G_5 but also by the upper part of G_4.

Terminal internode E_6 is somewhat elongated basally: 85 mm. as against 15 mm. for E_5. In its lower part it is the weakest of all the internodes, its resistance being about 20 gm. at this point. It should be noticed however, that in its upper part, it is actually stronger than E_6, as it offers a resistance of 750 gm. This observation confirms the conclusions of Blaringhem (3)

and Athanassof (2), based on anatomical studies, that maturation occurs sooner in two regions: first in the base of the culm, then in the part immediately below the ear, leaving the intermediate internodes, such as E_5, in an inferior stage of meristematic development.

It can be seen from Fig. 6 that the terminal internode presents the maximum length of weak tissues. Along 5 cm. of its length it offers less than 500 gm. resistance, and on the other 3.5 cm., less than 1000 gm. Since the diameter here is also minimum (1.5 to 2 mm. as compared with 4 mm. for internode E_3), it is obvious that the terminal internode is, at this stage, particularly weak. However it is also the most protected of all the internodes, for sheaths G_6, G_5, and G_4 are wrapped around it and maintain it erect. Curve G_6 shows the necessity of this multiple "wrapping." Even the complex of $G_6 + E_6$ offers at the base a resistance of 150 gm. only, and only 50 gm. at the false node. If however one considers in addition the sheath G_5, a total resistance of from 700 to 900 gm. is obtained (Fig. 6, G_5).

It is a general rule in the growth of grasses that tissues are not exposed to the light and open air until they have reached a high degree of maturation. Before this time they are covered by previously matured organs. The basal position of the growing zones of leaves and iuternodes is essential if the above condition is to be maintained.

In Fig. 6 it will be noticed that if the centre points of the sigmoid curves showing resistances of internodes are joined, an approximately straight line, reaching zero at the base of the ear, is obtained (E_5 being excepted). This fact shows an element of regularity which characterizes the culm both in the dynamic and static state (5).

As regards the accessory conditions affecting the measurements, hydration of tissues must be considered in any study of young culms, particularly during the period of growth. Fortunately, parts of the culm most susceptible to desiccation are also the best protected by "wrapping", so that a fresh culm may be studied many hours after collection with the assurance that its characteristics remain unaltered.

In studies on mature culms, necessarily dried, all exigencies of this type are entirely eliminated, and experiments may be performed at any time after collecting, providing the temperature and relative humidity remain constant during the whole series of measurements on which inter-comparisons are to be based.

C. Mature Culms

When elongation of the internodes is completed and the ear ripened, the tissues of the culm also reach maturity. The lower parts of the internodes become rigid, and finally the terminal internode reaches a condition represented by the graph (Fig. 7). The middle part is the most resistant to section (see also Fig. 8, curve A,I) and, from this maximum, the values decrease regularly toward the extremity, if the terminal reinforcement already discussed is neglected. The upper and lower ends often have about the same resistance, frequently only one-half or even one-third that of the middle part.

The crushing curve (Fig. 8, A,II) is, in general shape, the reverse of the cutting curve; its concavity is upwards and the maximum values are reached toward the extremities, particularly the upper one. While the resistance is only 250 gm. in the lower part, and 500 gm. in the upper part of the hollow region, it increases to over 2000 gm. at the top of the internode. At the extreme base the crushing strength increases to only 700 gm. for the bare internode near the nodal diaphragm, but it reaches over 2000 gm. when the measurement is taken at the level of the coarse, dried, false node, with the wrapping leaf included.

All these observations can be related to the flexion and compression stresses borne by the different regions of the internode, and to the varying thicknesses of their walls. In the adult stage the mechanical role of the sheaths becomes of less and less importance, and when it comes to the time of harvest, the internodes are stiff enough to remain erect without the help of the decayed and dried leaves.

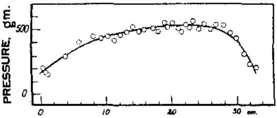

FIG. 7. *Resistance to section of an adult terminal internode of wheat. Apex to the right.*

Below the terminal one, the internodes are more resistant in direct relation to the greater stresses they must bear (Fig. 8, B). However in the basal part, resistance again decreases. Minima for section strength are reached at the extremities of the whole culm, even as they are reached at the extremities of the terminal internode alone. Of course mechanical stresses are similar whether one considers a whole culm or an isolated internode. The structure of the whole adult culm, from the point of view of anatomy, will be examined in detail in another publication.

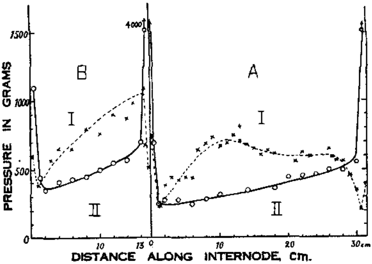

FIG. 8. *Cutting and crushing strengths of the terminal and subterminal internodes of a secondary culm of George Guthrie wheat (Central Experimental Farm, Ottawa): (I) graphs of cutting strength; (II) graphs of crushing strength; A, terminal internode; B, subterminal internode. All measurements on the bare internodes, with the apex to the right.*

To obtain measurements comparable from strain to strain* it is necessary to operate only on principal culms grown under the same conditions, and to take averages of many readings. Diameters, lengths of internodes, and the thicknesses of their walls must be measured at the same time. It is necessary also to eliminate

**Biological strains, i.e. varieties, etc.*

all imperfect culms, for any defect (injuries, insect galls, etc.) results in considerable changes in the mechanical properties of the tissues, generally by hardening them to a considerable extent.

A comparison of resistances to cutting and crushing is useful in a study of the probability of lodging in different varieties. As a matter of fact, however, factors other than mere resistance to a crushing or shearing stress affect lodging, in particular resistance to flexion and traction, and further work will have to be done on these matters before a complete understanding of the problem of lodging is obtained. These questions are not easy to investigate by reason of the difficulty of holding the tissues without injury, and also because of the practical impossibility of obtaining measurements at well determined and closely neighboring points. Thus, so far, determinations of cutting strength are superior to any other method in giving data more or less directly linked with other aspects of resistance.

In a practical application of the methods described above, the apparatus could be greatly improved by being rendered more or less automatic, and possibly self-recording, so that readings could be quickly and easily obtained from centimetre to centimetre along the length of a culm. This improvement would have the great advantage of eliminating the personal equation to a very great extent. Pressure would be applied very uniformly, section being determined by electrical contact between the blade and the platform. Such an installation would be more suited to an agricultural office than to a laboratory.

Summary

A simple method of measuring shearing (cutting) resistance of vegetal tissues has been described. Application of the method to cereal straws leads to the following conclusions.

The values obtained are obviously a function of several interrelated factors which will be separately investigated by other methods (histological, anatomical, physiological, etc.).

The method is useful, (i) in a study of the maturation of tissues in a growing stem, (ii) in comparisons of the characteristics of stems of different strains grown under similar conditions, (iii) in comparisons of the changes effected in the characteristics of stems of the same strain grown under different conditions.

It thus affords a means of analyzing the process of growth of a stem, the mode of protection of the young tissues (wrapping and unwrapping of the culm), and also of separating the effects of heredity from those of environment, as far as the question of the mechanical properties of the stem is concerned.

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STUDIES ON THE DEHULLING OF BARLEY KERNELS WITH SULPHURIC ACID AND ON THE INHERITANCE OF REACTION TO COVERED SMUT *USTILAGO HORDEI* (Pers.) K. & S. INFECTION IN CROSSES BETWEEN GLABRON AND TREBI BARLEYS[1]

By W. H. Johnston[2]

Abstract

The use of concentrated sulphuric acid for dehulling seeds of barley hybrids in order to induce infection by *Ustilago hordei* (Pers.) K. & S., involved complications which made exact analysis of the reaction of the hybrids impossible. Although the hull showed a high degree of resistance to decomposition by the acid and appeared to afford the embryo sufficient protection against acid injury, there were wide fluctuations in degree of emergence of plants from treated seeds, both of hybrid and parent material. Much of the mortality among treated seeds was due to mechanical injury incurred before treatment. There was considerable seedling injury from infection by *Penicillium* spp. This was probably augmented by unfavorable germinating conditions. Increased severity of infection by *U. hordei*, due to the removal of the hull resulted in extreme distortion of seedlings and consequent failure to emerge.

Genetic studies were made on the F_3 of reciprocal crosses of Glabron, highly resistant, and Trebi, moderately resistant to the form of pathogen used. Infection percentages exhibited by the parents were regained in some F_3 lines and the hybrids showed little tendency towards greater susceptibility than the parents. Segregation for reaction to covered smut was not sufficiently clear to establish the inheritance of this character.

Slight correlation was demonstrated between smut reaction and height of plant, but none between smut reaction and barbing of awns or earliness of heading. The development of strains combining earliness, smooth awns, and resistance to covered smut should present no great difficulty.

Introduction

According to reports on the "Prevalence of Plant Diseases in the Dominion of Canada" for the years 1927–33 (4, 5, 15), covered smut is probably the most widespread and destructive disease of the barley crop occurring in western Canada. In the province of Alberta for the five-year period 1927–31, this disease is designated as being common, destructive and causing important losses. The report of 1931 (4) shows 50% of the fields examined to have been infected, one exhibiting as high as 70% infection. Covered smut was present in 33% of the fields examined in both 1932 and 1933 (4).

Little or no work has been reported concerning the breeding of improved varieties of barley, resistant to covered smut. The early development of seed treatment measures for the control of the disease may account in part for the lack of investigation in this regard. Possibly more important has been the lack of a satisfactory inoculation technique for obtaining successful infections. Investigators have reported unsatisfactory infections from

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inoculating hulled* kernels (1, 2, 17). Dehulling by hand is impracticable when large populations are involved. The use of chemicals to dehull the kernels is still in the experimental stage and offers many difficulties.

The work reported in this paper summarizes the results of one phase of a general investigation conducted by the Department of Field Crops at the University of Alberta, Edmonton, during the years 1930–33. This general project had as its purpose the development of new, agronomically desirable, disease-resistant, smooth-awned varieties of barley, adapted to Alberta conditions (14).

Review of Literature

Environmental Considerations

The principal work regarding the effects of environment on the development of the covered smut disease in the barley plant is that of Faris (6, 7). Using controlled conditions, he was able to obtain high infection percentages of Hannchen barley over wide ranges of soil temperatures and acidity, and under moisture conditions generally existing in the soil at the time of seeding. Soil temperatures varying from 10–25° C. with 40–50% moisture content gave good infections at pH values of 5, 6, 7 and 7.8. In general, infection was inhibited by low soil temperature and moisture at time of seeding. Acid soils with 40 and 50% moisture content gave higher infections than did slightly alkaline soils. The vigor of the host plant was found to have little or no effect on the development of covered smut, although the growth conditions following infection may have a marked effect in that regard. The character of the soil was also found to play an important role in determining infection severity; quartz or builder's sand gave poor results while neutral potting soil gave normal infection.

Taylor and Zehner (18) have pointed out in the case of winter barley that higher percentages of infection with covered smut resulted from deep, than from shallow seeding.

Physiologic Specialization

Faris (6, 7) emphasized the fact that one of the important requirements necessary for high infection of covered smut in Hannchen barley was the use of inoculum collected from that variety. In a later paper (7), he demonstrated the presence of five physiologic forms by using four varieties of barley as differentials.

In covered smut collections obtained from Italy and several areas in the United States, Rodenhiser (17) distinguished seven physiologic forms on the basis of cultural characters. Two of these, a form obtained from Italy and one obtained from Minnesota, could also be distinguished by their pathogenicity on the varieties Lion and Himalaya.

Dehulling Studies

Tisdale (19) reported in the case of winter barley that the removal of the hull resulted in greatly increased infections. He removed the hulls by means

* *Throughout this paper the current practice is being followed of referring to "hulled" kernels, or varieties, as normal grain with the hull intact, and "dehulled" as kernels of hulled varieties from which the hulls have been removed artificially.*

of a scalpel and concluded that some simpler method of removing the hulls was necessary if extensive smut tests were to be carried out. Faris (7) also obtained greater infections when he used inoculated dehulled seed, but found that greatly reduced stands resulted.

Briggs (2) reported the results of extensive tests carried out at Davis, California, in the years 1919–21, with hulled seed of some 570 varieties and strains. Although the seeds were heavily inoculated with viable spores little infection resulted. Barley covered smut tests carried out at the University of Alberta in 1931 (1), with hulled kernels, confirmed the results reported by Briggs.

To obtain some simple method of dehulling the barley kernel, Briggs (2) studied the possibility of using sulphuric acid. It had been previously demonstrated by Brown (3) that the seed coat of barley was surprisingly resistant to this chemical. Briggs in his investigations varied both acid concentration and duration of treatment, but came to no definite conclusions regarding the most suitable treatment. Fortunately the hull over the germ was found to be thicker than elsewhere on the kernel, and a thin covering often remained over this region when the remainder of the kernel was completely dehulled. There appeared to be considerable variations in smut infection after apparently equal degrees of dehulling, due probably to the small numbers of plants used. He also found that low concentrations of acid for a long period of time gave infections comparable to high concentrations for short periods of time. A certain amount of corrosion of the kernels was noted when the more concentrated acid was used. It was also observed that complete dehulling was not necessary for good infection; in fact, a thin hull over the entire kernel seemed to give optimum results. He concluded that seed treated with sulphuric acid gave results comparing very favorably with seed dehulled by hand, and gave promise of usefulness in testing hybrid populations for smut reaction.

Experimental Studies

Materials and Methods

All genetic studies reported in this paper were carried out with reciprocal crosses between the varieties Trebi and Glabron. Trebi is a six-rowed, rough-awned variety resulting from a pure line selection from an importation, S.P.I. No. 15821, made in 1907 in the co-operative breeding experiments conducted by the United States Department of Agriculture and the Minnesota Agricultural Experiment Station at St. Paul, Minnesota (10).

Glabron is a six-rowed, smooth-awned variety, developed at the Minnesota Agricultural Experiment Station from a back-cross between Smooth-Awn (a selection from a cross between Lion × Manchuria) and Manchuria (11).

Trebi is moderately susceptible to infection with *U. hordei* in tests carried out at the University of Alberta, whereas Glabron is highly resistant (1).

The F_3 generation was used in all genetic studies reported, the purpose being to classify the F_2 plants on the basis of the infection percentages of the F_3 lines. Approximately 50 seeds were sown per 10-ft. row. Parental varieties were included at intervals of approximately 30 rows. The smut reactions of approximately 800 F_3 lines were tested.

The method of dehulling the kernels with sulphuric acid which was used in these studies will be described in detail later in this paper.

Inoculum consisting of a composite sample of chlamydospores collected from Velvet, Comfort and the two parent varieties was used in all inoculations. The method of inoculation consisted in shaking, in an envelope, a liberal quantity of the spores together with the dehulled seeds. In all cases the viability of the inoculum was tested before use by germinating the spores in water cultures.

The inoculated dehulled kernels were sown in a neutral soil with a temperature approximately 16° C. Owing to the dry condition of the soil it was necessary to seed two to two and a half inches deep instead of one to one and a half inches, the usual depth.

Dehulling with Concentrated Sulphuric Acid

Laboratory and field results.—To facilitate the dehulling of the barley kernels, a method of removing the hull with sulphuric acid was decided upon. Since no conclusive evidence as to the optimum strength and duration of treatment could be drawn from previous investigations, it seemed advisable to conduct a special study in an attempt to obtain further information on this problem.

To make possible the treatment of several samples of barley at one time, the apparatus shown in Fig. 1 was devised. This apparatus consisted of a tray composed of a wooden frame coated with paraffin, and four strands of copper wire serving as a support for two rows of fine bronze netted-wire baskets. The identity of the latter was kept by numbers inscribed on the frame. The method utilized in dehulling the kernels of the parental varieties will serve to illustrate the general technique adopted, while a modified method, used in dehulling the kernels of the F_2 hybrid plants, will be described later.

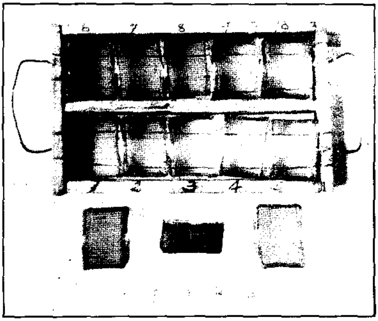

Fig. 1. *Immersion tray (including wire baskets) used in dehulling barley kernels with sulphuric acid.*

The tray containing the ten samples was immersed in the concentrated acid for a period of approximately five minutes, then removed and plunged quickly into cold water, in which the kernel lots were washed vigorously for about two minutes. To neutralize any acid that might still remain on the kernels the tray was next immersed in a concentrated solution of baking soda ($NaHCO_3$) for a period of two to three minutes. The kernels were then washed a second time in water before being placed on blotting paper to dry.

This treatment dehulled the majority of the kernels of the parent varieties fairly completely. A moderately thick hull remained over the embryo, whereas the amount of hull persisting over the remainder of the kernel varied from 25–50%. The kernels of Trebi appeared on the whole to have thicker or more resistant hulls than those of Glabron.

During the operation described above, it was discovered that if the kernels which had been treated for two to three minutes were washed in water and replaced in the acid for a few seconds when still wet, rapid and complete dehulling took place. That is to say, with the exception of the embryo region, over which a thin hull remained, the kernel was rendered more or less free of hull. This procedure appeared so promising in the light of laboratory germination tests, that it was decided to utilize it in dehulling the kernels of the F_2 plants. The technique employed in this modified treatment differed from that described above only in that the kernel lots were removed from the acid after a duration of two minutes, washed in cold water and replaced in the acid for the brief period of ten seconds. Some 50 to 60 lots of kernels could be treated and washed in an hour by this method.

Criticism may be made of this phase of the work, in that different methods were used in dehulling the hybrid and parental materials. Briggs (2) has pointed out that complete dehulling of the kernels is not necessary for optimum infection. With this in mind, it may be concluded that slight variations in the degree of dehulling will have little or no effect on the percentage of smut finally appearing in the plants produced from such kernels. Furthermore, it will be seen later in the paper that no observable differences in germination existed between the parental and hybrid lots of treated kernels. For these reasons, and to avoid confusion, no distinction will be made, in the remainder of this paper, between these two methods of dehulling barley kernels.

Examination of treated lots of kernels of both parental and hybrid material showed that the degree of dehulling of the kernels of individual plants was very uniform. The only kernels exhibiting variation were those whose hulls had been injured prior to treatment. On the other hand, observations on the variability in degree of dehulling amongst the kernel lots of different plants showed that, whereas no differences were apparent between the parental replicates, striking differences prevailed between kernel lots of different F_2 hybrid plants. The kernels of some plants showed an extreme condition of dehulling, while others showed a more moderate type, similar to that of the parents. These results indicate that the F_2 hybrids were segregating for thickness of hull or resistance to decomposition by acid.

It has been already pointed out that the dehulled kernels were sown in a comparatively dry soil. This condition was aggravated by the fact that no rain fell for a period of seven or eight days following seeding. That these factors checked normal germination was shown by the rapid and increased emergence which occurred after the first rain following seeding. Nevertheless, the final emergence of both hybrid and parental lines was disappointing. Large fluctuations occurred throughout the nursery. As the 50 kernels seeded per row were only estimated, it is impossible to state with accuracy the average reduction in stand of lines resulting from treated inoculated seed. However, a comparison of the stands resulting from treated seed with those resulting from untreated seed may be of interest. It was found that 65% of the hybrid lines, and 71 and 39% respectively of the Trebi and Glabron rows resulting from acid-dehulled seed, contained less than 25 plants. On the other hand, only 5% of 150 random hybrid lines (approximately 50 kernels per line) grown from normal hulled seed, contained less than 25 plants. Similar study of 50 random rows of each parent showed that only 4% of the Trebi rows and 2% of the Glabron rows had stands of plants below this limit.

Although the kernels of Trebi showed a greater thickness of hull than those of Glabron after acid treatment, it is interesting to note that the latter showed considerably less seedling loss. If any relationship existed between the degree of hull remaining on the kernel after acid treatment, and ability to emerge under field conditions, it was evidently masked by the activity of other agencies. The poor emergence of Trebi as compared with that of Glabron is thought to be due either to the characteristically slower germination of the kernels of the former, which would predispose them to attack from blue mold or other agencies, or to the greater susceptibility of this variety to the covered smut pathogen. It was expected that, since the kernel lots of the parent varieties showed a greater uniformity of dehulling than those of the hybrid lots, they would also show a correspondingly greater uniformity of field germination. The equally erratic germination of the hybrid and parental lines again leads to the belief that factors other than the degree of decomposition of the hull are responsible for the lack of field emergence noted.

Factors involved in seedling injury. An examination of the lines showing reduced stands revealed the fact that in a large number of cases germination had commenced, but owing to either the direct weakening effect of the acid or the indirect effect of dehulling, which exposed the young seedlings to a number of adverse environmental factors, the seedlings were unable to reach the soil surface. This inability to emerge was often accompanied by a condition of extreme distortion (Fig. 2, *B*). Faris (6) observed a similar distortion and lack of emergence of barley seedlings grown from hand-dehulled, inoculated seed. It seems reasonable to suppose that the removal of the hull unduly exposes the young seedlings during the early germination period, at a time when they are particularly susceptible to injury.

Other seedlings were found whose growth processes were early arrested by attack from blue mold fungi, *Penicillium* spp. (Fig. 2, *C*). The development of the root system seemed but slightly impaired, the greater injury occurring to the plumule. The stage of germination at which infection occurred varied widely, some seedlings being attacked just as the plumule was emerging through the coleoptile, others escaping until the plumule was well emerged.

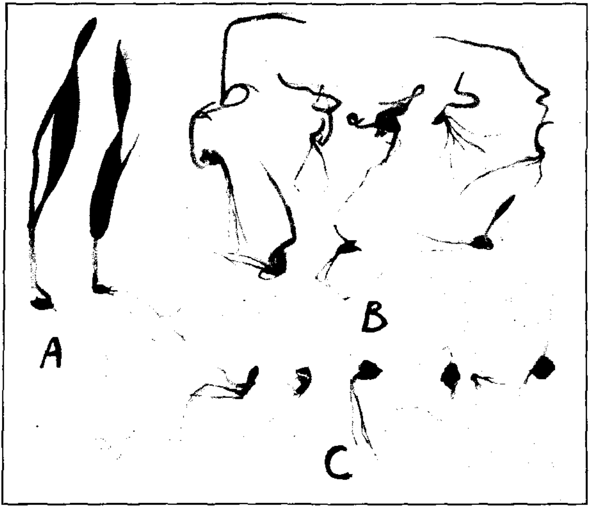

FIG. 2. *Injury to barley seedlings grown from kernels dehulled by sulphuric acid followed by inoculation with chlamydospores of Ustilago hordei. A. Healthy seedlings from non-dehulled uninoculated seed; B. Distorted seedlings from dehulled inoculated seed; C. Seedlings from seeds treated identically with B, showing distortion and stunting as a result of attack by mold fungi shortly after germination.*

Kernels showing no sign of germination were found in all the rows examined. This lack of vitality will be shown later to be due generally to seed coat injuries received prior to acid treatment.

In an endeavor to throw some additional light on the question of what factors were influential in reducing seedling emergence, two experiments were conducted in the greenhouse. Owing to the relatively small number of kernels used, certain minor variations between varieties occur; consequently only averages will indicate reliable trends.

Experiment I was conducted for the purpose of determining whether or not a higher activity on the part of the pathogen *U. hordei* could explain the

distortion of seedlings resulting from inoculated dehulled seeds. For this purpose uninoculated and inoculated lots of normal, hand-dehulled and acid-dehulled seeds of five varieties of barley were sown in sterilized soil. Fifty kernels, inoculated with a composite sample of smut spores, were used in each treatment. The temperature prevailing during this experiment was approximately 15° C. The results obtained are shown in Table I.

TABLE I

PER CENT SEEDLING EMERGENCE OF FIVE VARIETIES OF BARLEY, HULLED, DEHULLED BY HAND, AND WITH SULPHURIC ACID, WHEN INOCULATED WITH SPORES OF *Ustilago hordei*

Variety	Number*	Per cent emergence					
		Normal hulled		Dehulled by hand		Dehulled by acid	
		Un-inocu-lated	Inocu-lated	Un-inocu-lated	Inocu-lated	Un-inocu-lated	Inocu-lated
Bearer	C.A.N. 704	90.0	90.0	100.0	80.0	60.0	**53.3**
Glabron	C.A.N. 718	93.3	80.0	90.0	76.7	50.0	**40.0**
O.A.C. No. 21	C.A.N. 734	93.3	96.7	66.7	46.7	33.3	**23.3**
Saskatchewan Sel.	N.S.N. I-25-5	86.7	80.0	96.7	63.3	30.0	20.0
Trebi	C.A.N. 753	86.7	86.7	83.3	80.0	43.3	33.3
Average of the five varieties		90.0	86.7	87.3	69.3	43.3	34.0

* *C.A.N.—Canadian Accession Number.*
N.S.N.—Nursery Stock Number, University of Alberta.

The considerably lower germination percentage exhibited by the acid-dehulled seed, is again attributed to mechanical seed coat injuries received before acid treatment. The significance of this form of injury will become more apparent after considering the data contained in Table II. It will be noted also that, whereas there is little if any difference in the percentage emergence of uninoculated and inoculated hulled kernels, there is considerable difference in this regard in the dehulled series. The inoculated lots of kernels are invariably lower in emergence percentage. Examination of the soil supporting the inoculated lots revealed the presence of distorted seedlings. The results obtained here are especially significant in the case of hand-dehulled seed, and support the view that seedlings are injured by infection with *U. hordei*. An attempt to carry a number of the inoculated seedlings to maturity proved futile, as the conditions existing in the greenhouse were not conducive to normal heading. The spikes were small and in most cases sterile. Only two spikes exhibiting smutted spikelets were found. In view of the facts that viable inoculum was used, and that the temperature prevailing during the experiment was favorable, it can be safely assumed that considerable seedling infection actually did take place.

Experiment II was outlined to obtain some definite data on the ability of kernels, injured as a result of threshing operations, to germinate and emerge after acid treatment. Four varieties of barley were used in this experiment. Two lots of 50 seeds were made up from each variety, a random sample and one containing only those kernels showing some break of the hull over the embryo. It is important to point out that the latter sample contained no kernels with the embryo fully exposed. The results, as summarized in Table II, give ample evidence of the complete inability of kernels possessing mechanically damaged hulls to germinate after acid treatment.

TABLE II

PER CENT EMERGENCE OF SEEDLINGS FROM NORMAL AND MECHANICALLY INJURED KERNELS OF FOUR VARIETIES OF BARLEY AFTER TREATMENT WITH SULPHURIC ACID

Variety	Number*	Per cent emergence			
		Normal		Injured	
		Non-treated	Treated	Non-treated	Treated
Canadian Thorpe	C.A.N. 816	93	43	83	0
O.A.C. No. 21	C.A.N. 734	93	63	87	0
Saskatchewan Sel.	N.S.N. I-25-5	100	50	53	3
Wisconsin Barbless	C.A.N. 758	100	90	93	0
Average		97	62	79	1

* *C.A.N.—Canadian Accession Number.*
N.S.N.—Nursery Stock Number, University of Alberta.

Discussion

There was great variation in field germination of seed lots which received equal treatments with sulphuric acid. Precaution had been taken during the dehulling process to regulate the treatment so that a thin hull would remain over the germ of the kernels. Although minor variations in this regard were noted amongst the kernel lots of the hybrid lines, in no instances were seeds rendered entirely devoid of hull over this region. Furthermore, an examination of the embryos of kernels exhibiting the more extreme form of dehulling disclosed no noticeable injury. It appears, therefore, that under the conditions of this study, the hull of the barley kernel is quite resistant to digestion by sulphuric acid, and in the absence of complicating factors provides the embryo with ample protection. Studies now in progress (1) indicate that barley varieties differ considerably in thickness of hull or in resistance of the hull to acid decomposition. Trebi and Glabron were both found to belong to the group possessing the thicker or more resistant type of hull. This finding is in accord with the view expressed above.

A condition of the kernel that has been shown to be very effective in predisposing it to acid injury, is that resulting from seed-coat injuries over the germ, obtained as a result of threshing. The kernels used in the genetic

studies reported in this paper were threshed by a small head thresher. They were thought to be without extensive mechanical damage at the time of acid treatment, but subsequent and more careful scrutiny of other lots of kernels threshed by a similar method, showed the existence of numerous breaks of the hull over the embryo. Many of these were apparent to the naked eye, while others were observable only under a hand lens.

It is thought that these mechanical ruptures of the hull not only allowed the acid to come into contact with the embryo, but were instrumental in preventing complete removal of the excess chemical after treatment. It is conceivable that any delay in removing the acid might result in fatal injuries to the embryo. The low emergence percentages shown by the acid-dehulled kernels in Table I, are in all probability due to seed injuries received before acid treatment. The kernels in question were threshed by a rod-row thresher, which tended to leave the hulls in a greatly impaired condition. Here again the seriousness of these ruptures was not appreciated until after acid treatment.

Hurd (13) reports the presence of a natural rupture at the hilum end of the seed coats of threshed kernels of the Turkestan and Coast types of barley. This occurred whether the kernels were threshed by machine or by hand. Prolonged exposure of such kernels to saturated copper sulphate solution resulted in high mortality, whereas similar treatment of kernels left attached to pieces of the rachis resulted in no injury. She concluded that these fatalities occurred as a direct result of the chemical coming into contact with the embryo of the kernel by way of the natural rupture at the hilum. Since the spike structure of the two parental varieties used in this study appeared similar to that of the Coast variety, an experiment based on that of Hurd was conducted, to obtain data on the possibilities of sulphuric acid injury arising through the source just described. The duration of treatment in this experiment was identical with that used in dehulling the kernels of the hybrid lines. The results showed no difference whatsoever between the germination percentages of kernels treated while still attached to the rachis, and those treated in the threshed condition. This result is not surprising in view of the extremely limited exposure of the kernels to the acid. It may therefore be concluded that injury through the source reported by Hurd was not an important factor in reducing germination of the kernel lots used in the present investigation. However, it is significant that the mechanical seed coat injuries mentioned earlier in this section of the paper, arose from this natural rupture at the hilum. It is the existence of this weakness in the seed coat at the embryo end of the barley kernel that necessitates so much care in threshing kernels intended for chemical treatment.

The nature of the injury occurring from blue mold fungi was not apparent from field studies made. Hurd (13) has contributed valuable information in this regard in the course of a study on the susceptibility of injured wheat seeds to mold. Kernels with broken pericarps were germinated on blotters thickly sprinkled with spores of *Penicillium* spp. and *Rhizopus nigricans*.

She found that when the seed coat was ruptured over the endosperm 100% fatal infections occurred, whereas when the break occurred over the embryo the kernels proved immune. Also, injury from using copper sulphate and formalin as seed treatments, rendered previously immune seeds immediately susceptible, even perfect seed coats no longer acting as a protection. These observations of Hurd in regard to wheat would seem to explain admirably the nature of mold injury noted in this study. The acid treatment invariably exposed the endosperm at the distal end of the barley kernel, thus affording the mold fungus an excellent point of entrance. Furthermore, any injury of the embryo by acid would tend to render the kernel more susceptible.

It was felt that much less seedling injury from mold fungi would have occurred had conditions been more favorable for rapid germination. This supposition is supported by the evidence of Meyers (16) who found that mutilated corn seeds showed much less fatal injury from molds when conditions were favorable for germination.

Evidence also pointed to the fact that removal of the seed coat predisposed the young seedling to severe attack by the pathogen *U. hordei*. This was manifested in extreme distortion. A similar type of seedling injury has been reported by Tisdale and Tapke (20). These investigators, studying the possibilities of seedling infection as a result of inoculating hand-dehulled barley seed with spores of the loose smut fungus *Ustilago nuda*, showed definitely that the activity of the pathogen was responsible for distortion of seedlings noted in their work. They also found that dehulled inoculated seed sown three-fourths of an inch deep emerged more satisfactorily than similar seed sown one and one-half inches deep. Depth of seeding may also have been a complicating factor in the present study (see Materials and Methods).

Genetical

Inheritance studies. The reactions to the covered smut disease of the lines of nine crosses, involving Glabron and Trebi, were determined. Considerable variation in the severity of infection of individual plants was noted. This was due to the fact that the first spikes to emerge from the sheath tended to escape infection. Infected culms were found to be slower in heading than the healthy ones, and in a few cases the smutted spikes, rather than pushing their way through the top of the sheath, simply forced their way through the base. In one or two cases the peduncle bearing the smutted spike was badly distorted. In no instance did the leaves show smut sori as observed by Faris (6) in the case of severely infected plants.

All plants exhibiting any degree of infection were termed susceptible. Owing to reduced stands, the percentage infection was calculated for only those F_3 lines containing 15 or more plants. The lines were grouped in classes according to the percentage infection, in class intervals of 10%. A zero class was added to include all lines showing no infection.

The reaction of F_3 lines and parental varieties to the covered smut disease is given in Table III. The Glabron parent appeared highly resistant to the

collections of the pathogen used, and Trebi moderately so. The inherent resistance possessed by Glabron was really much greater than the data would indicate, as the majority of the rows contained only *one* partially infected plant. Parental rows of Trebi, on the other hand, often exhibited several totally infected plants. The overlapping of the ranges of infection percentages shown by the two parents, together with the wide range in this regard exhibited by Trebi, rendered impossible the working out of any definite mode of inheritance for covered smut reaction. Hybrid lines were found exhibiting the high degree of resistance of Glabron, while others showed susceptibility equal to that of Trebi. The resistance shown by the parental varieties was reflected generally in the progeny, there being little evidence of transgression towards greater susceptibility.

TABLE III

REACTION OF F_3 LINES AND PARENTAL VARIETIES TO INFECTION WITH *Ustilago hordei* IN RECIPROCAL CROSSES OF GLABRON AND TREBI

Parent or cross	No. of cross	Infection classes in percentage						
		0	1–10	11–20	21–30	31–40	41–50	51–60
Trebi	—	—	2	11	8	4	2	—
Glabron	—	9	24	2	—	—	—	—
Glabron × Trebi	6	2	14	10	6	1	1	—
	11	—	8	29	18	11	3	—
	12	13	49	37	10	6	3	2
	13	22	33	22	14	3	2	1
Trebi × Glabron	19	2	21	10	7	6	4	1
	20	4	11	24	10	8	4	1
	21	—	15	13	14	12	3	3
	23	—	6	11	17	10	9	1
	45	20	17	24	7	1	1	—
Total hybrid lines	—	63	174	180	103	58	30	9

Correlation studies. The importance of correlation studies in connection with the inheritance of disease reaction cannot be overestimated. This type of inheritance does not always lend itself readily to factorial analysis, and often it is only through establishing certain associations between the factors concerned in resistance and susceptibility, with those of some easily distinguishable morphological character, that it is possible to determine the number of factors concerned in a given disease reaction. Griffee (9) demonstrated in a striking manner the value of such linkage studies. He was able to show, by studying the reaction of F_3 lines to *Helminthosporium sativum* in relation to three other independently inherited characters, that at least three factors were concerned in resistance to this pathogen.

As part of the more general program alluded to earlier in this paper (14), data were compiled on the mean number of days from emergence to heading, as well as on the mean height of each F_3 line of the two crosses 12 and 21 (see Table III for parentage of crosses). Barbing of awn was also studied in these two crosses as well as in cross 45.

The results of these studies may be briefly summarized. Barbing of awns was found to be governed by two factor pairs which could be explained on the basis of epistasis. Rough, intermediate-smooth and smooth-awned plants were obtained in the ratio of 12 : 3 : 1 in the F_2. Earliness of heading was shown to be an inherited character and could best be explained by assuming two main complementary factors. Transgressive segregation for earliness and lateness occurred in both crosses studied. The coefficient of variability of the F_3 lines in regard to mean number of days from emergence to heading was 7.37 ± 0.27 and 7.55 ± 0.35 for crosses 12 and 21 respectively. The coefficient of variability of the parental varieties, on the other hand, ranged from 2.11 ± 0.45 to 3.56 ± 0.60. Height of plant was found to be readily influenced by environment. This was particularly evident in cross 21, in which the parents showed coefficients of variability of 7.00 ± 1.49 and 5.59 ± 1.19, as compared with the value 8.42 ± 0.39 shown by the hybrid lines. More positive evidence regarding the heritable nature of this character was obtained from cross 12. The F_3 lines of this cross showed a coefficient of variability of 7.74 ± 0.29, as compared with 4.88 ± 0.82 and 4.71 ± 0.79 of the parent varieties.

In order to determine whether any association existed between the different combinations of smut reaction, height of plant and number of days to heading, simple correlation coefficients were calculated (12). These values may be found in Table IV. High correlations exist between mean height of plant and mean number of days to heading. Smaller but significant positive correlations exist between covered smut reaction and both mean height of plant and mean number of days to heading in cross 21, as well as between smut reaction and height of plant in cross 12. The value $r = .193 \pm .059$ obtained between mean number of days to heading and covered smut reaction in cross 12 is of extremely doubtful significance. To determine the causal relationships existing between these variables, partial correlations were calculated (8). These data are also presented in Table IV.

TABLE IV

SIMPLE AND FIRST ORDER PARTIAL CORRELATIONS BETWEEN SMUT REACTION AND BOTH MEAN HEIGHT OF PLANT AND MEAN NUMBER OF DAYS FROM EMERGENCE TO HEADING

—	Number of cross	Simple correlation coefficients: Mean number of days to heading (2)		Simple correlation coefficients: Per cent covered smut infection (3)		First order partial coefficients
Number of cross		12	21	12	21	
						$r_{12.3}$ cross 12 $+ .715 \pm .026$ cross 21 $+ .688 \pm .034$
Mean height of plant (1)	12	$.728 \pm .025$	—	$.266 \pm .060$	—	
	21	—	$.737 \pm .030$	—	$.498 \pm .065$	$r_{13.2}$ cross 12 $+ .187 \pm .062$ cross 21 $+ .362 \pm .075$
Mean number of days to heading (2)	12	—	—	$.193 \pm .059$	—	
	21	—	—	—	$.366 \pm .075$	$r_{23.1}$ cross 12 $- .003 \pm .061$ cross 21 $- .002 \pm .086$

The relationship between mean number of days to heading and mean height of plant appears to be little influenced by degree of smut reaction. On the other hand, when the variable mean height of plant is held constant, no association whatsoever exists between mean number of days to heading and degree of smut reaction. With mean number of days to heading held constant, a small but significant positive correlation is found between degree of smut reaction and mean height of plant in cross 21, and one of doubtful significance in cross 12. However, it is possible that limited genetic linkage exists between the factors concerned in smut resistance and those concerned in height of plant.

The correlation ratio (12) was employed to determine a possible relationship between barbing of awns and degree of smut reaction. The value, $\eta = .030 \pm .043$ obtained, is indicative of an entire lack of association between these characters.

Conclusions

An adequate and uniform population is essential for any genetic investigation. It is evident that the stands resulting from kernel lots dehulled with concentrated sulphuric acid, were too variable to be of great value in testing the reaction of a hybrid population to *U. hordei*. Evidence indicated that under the conditions of this study the hull of the barley kernel was quite resistant to the acid, and when free from mechanical injuries offered the embryo ample protection. A complex of factors was found responsible for the lack of field emergence noted in this study. Notable among these was the acid treatment of kernels possessing hulls broken in the embryonic region. After dehulling, increased pathogenic activity of the covered smut fungus, together with attack by blue mold fungi, resulted in a further toll of the young seedlings. Unfavorable germinating conditions were thought to have aggravated the general situation.

The reader should not be unmindful of the fact that only concentrated acid was used. Hence, a too hasty condemnation of the chemical would not be justified. However, under the conditions of this investigation, it is unlikely that a less concentrated form would have proved any more satisfactory; since, to obtain the same degree of dehulling, the kernels would necessarily have to be treated for a greater period of time. This would have afforded the acid even a greater opportunity of coming into contact with the embryo, had any rupture of the hull been present. However, it is possible that the degree of dehulling attained in the present study was more complete than was necessary for good infection. In that event a less concentrated solution of acid might have proved more satisfactory. It was felt, furthermore, that even the concentrated acid might have proved more or less successful had the precaution been taken to thresh all kernel lots by hand, so that the seed coats would be free of injury. Again, there is always the possibility that further research will bring to light chemicals more satisfactory for dehulling

barley kernels than sulphuric acid. At this juncture the question might well be asked; Is further investigation of the use of chemicals as dehulling agents warranted?

Surveys have shown the existence of comparatively high natural infections of covered smut in farmers' fields. These cannot always be attributed to superior environmental conditions in these fields, or to the presence of a more virulent form of the pathogen, since such factors have been readily controlled by investigators. The most plausible explanation would appear to lie in the practice of close threshing followed by many farmers, which tends to leave the barley kernels in a more or less dehulled state, descriptively termed "skinned". In this connection Faris (7) makes the following statement: "I have no doubt, however, that in farm practice, injury to the hull over the seed embryo may play a part in the amount of loss from this barley smut. In fact, I have received field-threshed barley with a considerable portion of the seed as effectively dehulled by close threshing as could be done by hand." The writer has also seen barley samples so badly "skinned" as to compare favorably with hand-dehulled seeds in the completeness of dehulling.

In the light of the above observations, it would appear that the development of some system of close threshing would possibly prove more fruitful as a means of inducing covered smut infection, than continued research with chemicals. Tisdale (19) has suggested that scarifying the seed before smutting might possibly give as good results as removing the hulls by hand. One obvious advantage of dehulling barley kernels in some way other than by chemicals, lies in the fact that machine-threshed seed could be safely used.

Doubtless, a certain percentage of seedling loss would still occur from seed mutilation and from the activity of other agencies discussed earlier in the paper. These losses could be readily offset by increasing the number of kernels sown per row.

Genetic studies reported in this paper, while failing to reveal any definite mode of inheritance of covered smut reaction, clearly demonstrate its heritable nature. Since no genetic linkages appear to exist between covered smut reaction and either earliness of heading or barbing of awns, no great difficulty should be experienced in developing new homozygous strains possessing the desired combination of earliness, smooth awn and smut resistance.

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ON THE INVASION OF ROOTS OF *MEDICAGO* AND *MELILOTUS* BY *SCLEROTINIA* SP. AND *PLENODOMUS MELILOTI* D. AND S.[1]

By M. W. Cormack[2]

Abstract

The invasion of roots of *Medicago* and *Melilotus* by *Sclerotinia* sp. and *P. meliloti* was studied, with particular reference to the effectiveness of wound cork in arresting the progress of these pathogens. Both pathogens are apparently capable of penetrating through the uninjured external cork covering of the roots and also through any subsequently formed wound cork layers. Killing of the root tissues and retardation of wound cork development in advance of hyphal invasion was caused by both pathogens. No explanation can at present be offered as to why the advance of *P. meliloti* is checked and that of *Sclerotinia* sp. is definitely slower after good growth begins in the spring.

Recent studies of Sanford and Cormack (9, 10) have shown that *Sclerotinia* sp. and *Plenodomus meliloti* are destructive root-rotting pathogens of *Medicago* (alfalfa) and *Melilotus* (sweet clover) in Alberta, especially following the winter dormancy period. During the summer, *P. meliloti* ceases to be pathogenic, while *Sclerotinia* sp., although still capable of rotting the roots, causes far less damage than it does in the early spring. It was thought that the plants might lack the ability to form wound cork as they emerge from dormancy, and that this might account for their increased susceptibility at that time. Cormack (3) demonstrated that wound cork was retarded in roots of alfalfa and sweet clover which had been frozen, and also that it formed very slowly at low soil temperatures, but fairly rapidly during summer, when the temperature is higher.

The present study concerns mainly the relation of wound cork to the invasion of roots by the two pathogens indicated.

Materials and Methods

Inoculation of Plants

In this study four varieties of alfalfa and four of sweet clover were used, each one differing in degree of winter hardiness. The tests were made under both greenhouse and field conditions. In most experiments the roots of vigorous plants grown from seed planted in the field in June were inoculated the following September. In one case vigorous plants were transplanted into boxes, hardened off for a week, then inoculated with *P. meliloti* and artificially frozen at –5° C. for 3 days, after which they were held in a greenhouse at a temperature of approximately 18° C. More satisfactory infection was obtained in the field than under greenhouse conditions. The two pathogenic cultures used were isolated from diseased sweet clover roots. The culture of *Sclerotinia* sp. has not been definitely identified, but Dr. H. H.

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Whetzel, who has examined it, has suggested that it may be an undescribed species closely related to *S. minor*. *P. meliloti* has been described by Sanford (9).

In preparing the roots for treatment the soil was removed from one side of each tap root to a depth of 3–4 in., and the surface left clean. The roots of one set of plants were uniformly wounded with a sharp scalpel at the point of inoculation. The roots of an equal number of plants of each variety were treated similarly, except that they were not wounded. For those series inoculated a small amount of oat hull culture of the pathogen was placed on a thin piece of absorbent cotton and packed firmly against each tap root. At regular intervals after treatment, replicate sets of plants of each variety and treatment were removed from the soil for histological examination. The extent of wound cork development beneath lesions and wounds, and the severity of infection were estimated numerically, using values from 0 to 10.

Histological Technique

The inoculated, wounded, or otherwise treated portion of each root to be studied was fixed, infiltrated with paraffin, and sectioned about 10 μ thick. The mounted sections were stained overnight in a 95% alcoholic solution of Sudan III, in a 0.5% aqueous solution of acid fuchsin for about 5 min., and finally in a 0.5% aqueous solution, consisting of 9 parts of light green and 1 part of iodine green, for about 10 min., washing between stains. Good differentiation of cork and other tissue was secured, the cork cells staining orange, the diseased tissues and mycelium pink to red, and the normal tissues blue.

Experimental Results

Observations on Root Structure

Roots of alfalfa and sweet clover have essentially the same structure. The primary cortex of the early seedling stage soon becomes stretched and ruptured by secondary growth, sloughs off, and is replaced by a permanent cork covering which is complete when the plants are about four weeks old. This external cork layer is generally three to five cells wide and forms a practically complete protective covering for the root, except where branch roots pass out and lenticels occur. Such openings are usually protected by deeper lying cork layers, which are at least two cells wide.

Root Penetration by the Pathogens

Sclerotinia sp. Wounded and non-wounded roots were attacked with approximately equal severity, indicating that this pathogen is apparently capable of penetrating through the external cork covering of the roots. Representative results, which are given in Table I, show that this was true with varieties of both alfalfa and sweet clover, although the latter were more severely attacked. Histological examination showed many cases of what appeared to be direct penetration of the uninjured cork wall. The growing hyphae usually accumulated in close contact to the root and gradually broke through the protective cork covering (Plate I, A and B), after which they

surged through into the underlying tissues (Plate I, C). In some instances the underlying cells were disorganized and a dark staining substance was formed below the cork covering before it was completely penetrated by the hyphae (Plate I, A and B). These observations indicate that penetration may have been accomplished partly by hyphal pressure and partly by enzymatic action, as described by Lüdtke (6).

TABLE I

INFECTION CAUSED BY *Sclerotinia* SP. IN WOUNDED AND NON-WOUNDED ROOTS OF *Medicago* AND *Melilotus*, 10 DAYS AFTER INOCULATION

Variety	Infection rating*	
	Wounded roots	Non-wounded roots
Medicago		
Grimm	1.0	1.5
Cossack	1.0	1.5
Baltic	2.0	1.5
Liscombe	1.5	1.0
Melilotus		
White Blossom	9.0	8.5
Arctic	7.0	7.5
Maccor	9.0	7.5
Yellow Blossom	6.0	5.5

**Average numerical rating of four replicates.*

Plenodomus meliloti. This pathogen also attacked non-wounded roots as readily as those which had been wounded. Histological observations indicated that it penetrated the external cork covering by mass hyphal action, as described by Conant (2). Dense masses of hyphae were often observed in close contact to the disintegrating cork layer (Plate I, F), and frequently pycnidia of the fungus were embedded in the remains of the cork layer after the hyphae had entered the roots (Plate I, G).

Progress of the Pathogens Through the Root Tissues

Sclerotinia sp. After penetrating the roots of either alfalfa or sweet clover, this pathogen often spread rapidly, particularly in the tissues of sweet clover. The hyphae passed both through the cells and between the cells (Plate I, E), and usually made greatest progress parallel to the main axis of the root. Frequently the root tissues were at least partly killed several cells in advance of the pathogen. This was indicated by the staining characteristics and disorganized appearance of the cell contents of the tissue bordering healthy parts (Plate I, D).

An interesting feature was the fact that a complete layer of wound cork seldom occurred in advance of invasion. Such wound cork as formed did not seem to offer appreciable, if any, resistance. In Table II it will be observed that although the roots of sweet clover were attacked much more severely than those of alfalfa, the amount of wound cork formed in the non-inoculated roots was approximately the same. In the inoculated roots, wound cork was severely inhibited or failed to appear when invasion was in progress. Thus, on the basis of the data obtained in this and previous studies (3), it would seem that wound cork does not play a significant role in this root rot, either in the early spring or later in the season. It should be mentioned that in the case of alfalfa it was found, by experiments carried out in midsummer, that sometimes wound cork, several cells in thickness, did

form beneath lesions that had made but little progress. Since *Sclerotinia* sp. seldom produced extensive lesions on alfalfa roots (Tables I and II), the presence of this wound cork was not considered very significant in connection with the development of disease under field conditions.

TABLE II

INFECTION AND WOUND CORK DEVELOPMENT IN ROOTS OF *Medicago* AND *Melilotus*, NON-INOCULATED AND INOCULATED WITH *Sclerotinia* SP. AND *P. meliloti*

Variety	Treatment	*Sclerotinia* sp.*		*P. meliloti*†	
		Infection	Wound†† cork	Infection	Wound†† cork
Medicago					
Grimm	Inoculated	1.3	0.3	3.2	0.0
	Non-inoculated	0.0	3.1	0.0	3.2
Cossack	Inoculated	1.2	0.2	3.3	0.0
	Non-inoculated	0.0	2.8	0.0	4.0
Melilotus					
White Blossom	Inoculated	8.8	0.0	2.2	1.5
	Non-inoculated	0.0	3.8	0.0	3.8
Arctic	Inoculated	7.3	0.0	1.9	1.0
	Non-inoculated	0.0	4.1	0.0	3.8

**Field experiment, 10 days after treatment.*

†Laboratory experiment with plants artificially frozen at −5° C. for 3 days, 13 days after treatment.

††Average numerical rating of four replicates.

Plenodomus meliloti. The hyphae of this pathogen were frequently seen between the cells and also inside of cells of invaded tissues. There was even more marked killing of the tissues in advance of the hyphae than with *Sclerotinia* sp. Also, wound cork was severely suppressed, and in many instances failed to appear even at favorable soil temperatures. In spite of this, the lesions were arrested during early June, when the growing season started. Hence, as in the case with *Sclerotinia* sp., wound cork evidently is not a factor in arresting the progress of the disease. In place of wound cork, a dark brown to black deposit of unknown composition usually appeared between the lesion and the sound tissue of actively growing plants, particularly in older lesions.

Toxic Effect of Pathogens

It has been shown that both *Sclerotinia* sp., and *P. meliloti*, particularly the latter, often kill and disorganize the root tissues in advance of the invading hyphae. The presence of either pathogen also severely retards and often completely inhibits wound cork development in the adjacent tissues. Also, in connection with the penetration of *Sclerotinia* sp. a dark staining substance was sometimes observed below the disintegrating cork covering

of the root. Since these phenomena were observed only in the presence of the pathogens, it is conceivable that they are caused by products of metabolism toxic to the root tissues.

The nature of the toxic substances concerned is unknown, but it was thought that the pH relationships might be important. Herklots (4) found that acidity up to pH 4.6 promoted the formation of cork in the potato tuber, but delayed its subsequent suberization, while a reaction of pH 7.5 and higher promoted suberization, but retarded meristematic activity. The reaction of alfalfa and sweet clover roots invaded by *Sclerotinia* sp. and by *P. meliloti*, and of the healthy root tissues of similar plants, was determined electrometrically with a quinhydrone electrode, using a small amount of distilled water to make a solution of the tissue. The average reaction of healthy roots of alfalfa and sweet clover was pH 6.2 and pH 6.4, respectively. Roots of alfalfa invaded by *Sclerotinia* sp. gave a reaction of pH 5.4, while those of sweet clover had a pH value of 4.9. There was no significant change in the reaction of roots invaded by *P. meliloti*. Zeliff (13) states that the optimum reaction for growth of *S. sclerotiorum* is pH 3.2, and Sanford (9) states that the optimum for *P. meliloti* is pH 6.2. Thus, it would appear that the reaction of the invaded tissues depends to some degree on the optimum for growth of the pathogen concerned.

Discussion

Several important points in the foregoing data may be emphasized, and their relation to the problem evaluated. In the first place, both pathogens appeared to penetrate the root covering which consists of several layers of well suberized cork cells. Secondly, wound cork was, as a rule, practically suppressed in the presence of either pathogen, and there was often killing of the tissues in advance of the invading hyphae of both. Finally, there is the question as to why the advance of *P. meliloti* is checked when the plants regain their summer vigor during June or later, while progress of *Sclerotinia* sp., although retarded, is not completely checked.

It is common knowledge that pathogenic fungi penetrate with difficulty and are frequently arrested by adequately suberized cork layers. Priestley (8) found that the suberin impregnating cork walls was unmixed with cellulose, and could not be decomposed by the ordinary hydrolyzing enzymes excreted by parasitic fungi. Jones (5) observed that *Actinomyces scabies* was able to pass through the suberized barriers in the potato tuber by growing in the unsuberized middle lamella of the cell walls. Matsumoto (7) reviewed most of the important early work on cell penetration by pathogenic fungi. His studies showed that *Rhizoctonia solani* penetrated directly through the cuticle of plants and that this was chiefly a mechanical process. Brown and Harvey (1) concluded that pathogenic fungi could penetrate the plant cuticle only in a purely mechanical way. On the other hand Lüdtke (6) found that the penetration of pathogens into host plants was effected partly by mechanical means and partly by enzymatic action, which conclusion is supported by the

recent studies of Thomas (11) on the parasitism of *Armillaria mellea*. Conant (2) concluded that the enzymes produced by the aggregated hyphae of *Thielavia basicola* could break down the suberized tissues of tobacco roots, allowing the fungus to break through the weakened cork walls. Our infection studies and histological observations have given unmistakable evidence that *Sclerotinia* sp., and *P. meliloti* can penetrate the cork covering of alfalfa and sweet clover roots in the absence of either lenticels or wounds. While there are indications that the hyphae of these pathogens excrete enzymes and also evidence of mass hyphal pressure in penetrating the cork layers, a decision as to which is more effective cannot be made on the data given.

With regard to the observed suppression of wound cork and other abnormal effects, a number of fungi are known to have a toxic effect on plant tissues in advance of hyphal invasion. Tubeuf and Smith (12), in discussing *S. sclerotiorum*, quote from an early paper by De Bary as follows: "The dead parts of the plants serve as nutriment to the fungus, which makes its way into the tissues and causes death of cells in direct contact or immediate neighborhood. The deadly fluid separated by the fungus contains, as an essential constituent, an enzyme soluble in acid solutions and capable of dissolving the cell walls; also a number of imperfectly known organic and inorganic acids and salts, amongst which oxalates can certainly be proved". As mentioned previously, the hyphae of both *Sclerotinia* sp. and *P. meliloti* had a definite effect on the tissue in advance of invasion. If this is due to metabolic products which are quickly or even slowly lethal to normal cell function, it is conceivable that meristematic activity necessary to the initiation of wound cork might be greatly retarded or definitely suppressed. The reaction of the invaded tissues would seem to be of doubtful importance in relation to the toxic effects of the pathogens, since the change in reaction observed did not differ greatly from normal.

There is no doubt that alfalfa and sweet clover are most susceptible to both *Sclerotinia* sp. and *P. meliloti* following the winter dormancy period, and also that these plants are not attacked by the latter pathogen after the growing season has started. While mechanical injury may increase infection in certain instances, this study has demonstrated that penetration is by no means dependent on this type of injury. At the low soil temperatures which prevail during April and May, wound cork forms very slowly, and even if there were no other complicating factors, such as the suppression of it in the presence of the pathogen, it is doubtful if the progress of these pathogens would be materially affected. Possibly later in the season at higher soil temperatures wound cork might play a role in checking *Sclerotinia* sp. during the early stages of invasion, but, as Thomas (11) suggests in the case of *Armillaria mellea*, such wound cork might not be effective. However, should such lesions become extensive, wound cork would be immediately arrested, and become an unimportant consideration. Consequently, the general conclusion is that in Alberta during April, May, and possibly June, wound cork plays a negligible role, while during summer months it may aid

in arresting incipient lesions caused by *Sclerotinia* sp. or by certain weak parasites, which may not excrete substances unfavorable to wound cork development.

No explanation can be offered as to why lesions caused by *P. meliloti* during early spring cease to make further progress during summer. At least wound cork is absent or negligible in quantity. Whether at higher temperatures the dark brown to black deposit bordering the lesions becomes a barrier, or whether certain physiological conditions which occur only in the more actively growing plants are responsible, is not clear at this time.

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ISOLATION OF S AND R TYPES BY CATAPHORESIS[1]

By G. B. Reed[2] and B. G. Gardiner[3]

Abstract

It is shown that by taking advantage of the differences in cataphoretic velocities it is possible to separate S and R types of certain acid-fast species of bacteria.

It has been previously demonstrated (Reed and Gardiner (7)) that there is a definite difference in electrophoretic charge between the S and R forms of *Mycob. leprae* described by Reed (6). This difference was correlated with differences in their isoelectric points and their tendency to be agglutinated in acid suspensions. Similar differences in the cataphoretic speed of S and R types of the tubercle bacillus have been shown by Kahn and Schwarzkopf (3). This is in line with the extensive investigations of Falk (2), Chapman (1), Rosenow (8) and others on different species of bacteria.

The work of Kligler and his colleagues (4, 5) shows that various viruses suspended in buffered solutions can be recovered at the anode after cataphoresis, apparently being carried there by the associated proteins. This suggested that a similar method might serve to separate S and R forms, at least in cases where there exists a demonstrable difference in the cataphoretic rate.

An apparatus was set up, as shown in Fig. 1, consisting essentially of two chambers, *A*3 and *C*3, which were filled to the same level with physiological saline and connected by a rather large siphon. Each of them was connected by a potassium chloride agar bridge to another chamber, and eventually to a non-polarizable electrode. In this case copper electrodes in copper sulphate solution were used. These were connected with the 120-volt, direct-current line. An impressed voltage of 220 resulted in enough heating to kill the bacteria eventually while a voltage less than 120 resulted in a migration too slow to give satisfactory results.

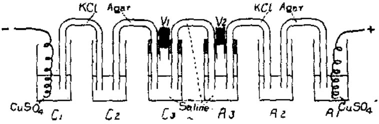

Fig. 1. *A cataphoretic chamber for the isolation of R and S forms of bacteria, A1 and C1, Cu–$CuSO_4$ electrodes connected with KCl agar bridges to KCl chambers A2 and C2 which in turn are connected by agar bridges to cathode chamber C3 and anode chamber A3 communicating by a siphon. Vents for introducing organisms are shown at V1 and V2.*

The chambers *A*3 and *C*3 were autoclaved, together with the physiological saline and the connecting siphon. Openings for the agar bridges were plugged during this operation. Sterile potassium chloride agar bridges were then inserted and the assembled apparatus tested for sterility.

[1] *Contribution from the Department of Bacteriology, Queen's University, Kingston, Ontario, Canada.*
[2] *Professor of Bacteriology, Queen's University.*
[3] *Holder of a Studentship under the National Research Council of Canada.*

Pure S and R types of *Mycob. leprae*, 513, were grown on Petragnani's medium. After seven days' growth suspensions of equal density of the two types were made in physiological saline. Equal amounts of these suspensions were then mixed in a sterile tube and centrifuged. The supernatant was then removed, and the organisms resuspended in a few drops of physiological saline. One drop of this mixed and concentrated suspension was added with a sterile Pasteur pipette to the chamber *C3* through the vent *V1*; at the same moment, in order to eliminate any slight siphoning action, one drop of sterile saline was added to the chamber *A3* through the vent *V2*. At intervals both before and after the current was turned on, samples were removed through the vents by means of a sterile platinum loop and streaked directly on Petragnani's medium. Colony counts of these cultures were made after seven days' growth.

Table I shows a typical set of results. The S organisms appeared at the anode chamber first, in something less than 30 min., while the R organisms did not arrive there until after about 45 min. Before the current was turned on, a culture from the cathode chamber showed approximately equal numbers

TABLE I

EFFECT OF CATAPHORESIS IN SEPARATING S AND R TYPES OF *Mycob. leprae, 513*

Time exposed to current	Type of colonies from cathode	Type of colonies from anode
0	R and S, approx. equal	Sterile
15 min.	R and S, approx. equal	Sterile
30 min.	R and S, approx. equal	S only, several hundred colonies
45 min.	R and S, approx. equal	Several hundred S, one R colony
1 hr.	Mixed S and R	Mixed S and R
2 hr.	Mixed S and R	Mixed S and R
4 hr.	Mixed S and R, but somewhat more R	Mixed S and R
12 hr.	118 R colonies, 20 S colonies	Mixed S and R

TABLE II

EFFECT OF CATAPHORESIS IN SEPARATING S AND R TYPES OF PETROFF'S STRAIN OF *Mycob. tuberculosis avium*

Time exposed to current	Type of colonies from cathode	Type of colonies from anode
0	R and S, approx. equal	Sterile
15 min.	R and S, approx. equal	Sterile
30 min.	R and S, approx. equal	18 S colonies
1 hr.	R and S, approx. equal	Several hundred S, 3 R colonies
2½ hr.	Mixed S and R	Mixed S and R
5 hr.	Mixed S and R	Mixed S and R
10 hr.	Mixed S and R	Mixed S and R
20 hr.	Mixed S and R	Mixed S and R

of S and R colonies, while after 12 hours' treatment the R colonies outnumbered the S by six to one. This result amply confirms the previous findings as to the cataphoretic speed of the two types.

By the same technique, similar experiments were performed using R and S strains of Petroff's avian tubercle bacillus. A typical set of results is given in Table II. The general trend was similar to the results with *Mycob. leprae* but the movement toward the anode was slightly slower. This is in agreement with the finding that the cataphoretic movement of the avian tubercle bacilli is slower than the *Mycob. leprae*.

Although the results render it highly improbable that siphoning or similar current action had any appreciable effect, a control was run to eliminate this possibility. This was done by placing the organisms in the anode chamber *A*3 instead of cathode chamber, *C*3. On examination, cultures from *A*3 made at varying intervals up to 24 hours all showed mixed S and R colonies, while those from *C*3 were consistently sterile.

Conclusions

An apparatus has been described, resembling one used by Kligler in virus work, in which a mixture of S and R forms of *Mycob. leprae* and avian tubercle bacilli were separated by taking advantage of the differences in cataphoretic velocity of the two types.

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ON THE ACOUSTIC RADIATION FIELD OF THE PIEZO-ELECTRIC OSCILLATOR AND THE EFFECT OF VISCOSITY ON TRANSMISSION

PART II[1]

BY L. V. KING[2]

Abstract

Numerical data on the distance of transmission of sound in sea water from a 10-in. piezo-electric oscillator are discussed in the light of theoretical results obtained in a previous paper. It is shown how by the principle of similitude the chart for transmission at optimum wave-length calculated for a 50-watt, 60 cm. oscillator can be used for a transmitter of any given diameter and output. A comparison with some experiments of Boyle's points to the fact that at supersonic frequencies, in the neighborhood of 100,000 cycles, a considerably higher coefficient of viscosity than that obtained by flow methods must be used.

Very few data on the performance of actual piezo-electric oscillators have been published. The writer is indebted to Dr. R. W. Boyle for numerical data and valuable criticism enabling some comparison to be made between observation and the numerical data employed to illustrate formulas derived in this paper.

The coefficient of viscosity of sea water was measured by the capillary flow method and was found to be 0.017 at 7.3° C. Interpolation between the values $\mu_0 = 0.0181$ at 0° C. and $\mu_{20} = 0.0101$ at 20° C., calculated from tabulated values for sodium chloride solution of density 1.024, gives $\mu_{7.3} = 0.016$, in good agreement with Dr. Boyle's determination.

In former unpublished tests carried out in sea water it was found that a 10-in. disk at a frequency of 100,000 gave at $2\frac{1}{2}$ miles a moderately strong signal estimated from reception by a piezo-electric receiver to correspond to a displacement amplitude of 3×10^{-10} cm. in the water. This enables us to estimate the velocity amplitude and output of the transmitter. If we denote by $|\xi|$ the displacement amplitude in the central beam, we easily deduce from the formulas of Section 11 (3) that

$$|\xi| = \frac{1}{2}\frac{a^2}{c}\,|\dot{x}|\,\frac{e^{-\kappa' r}}{r}\ . \tag{1}$$

Using the above data supplied by Dr. Boyle for the 10-in. oscillator transmitting at 100,000 cycles, we have, $a = 12.8$ cm., $\kappa_0 a = 53.9$, $|\xi| = 3 \times 10^{-10}$ cm., $r = 2\frac{1}{2}$ miles $= 4 \times 10^5$ cm., $\mu_7 = 0.017$, $c = 1.49 \times 10^5$, $\kappa_0 = \omega/c = 4.20$.

From Equation 68 (3) we have

$$\kappa' = \frac{2}{3}\frac{\mu\kappa_0^2}{c\rho_0} = 1.30 \times 10^{-6}\ .$$

[1] *Manuscript received July 26, 1934.*
Contribution from the Department of Physics, McGill University, Montreal, Quebec, Canada.
[2] *Macdonald Professor of Physics, McGill University.*

Solving for $|\dot{x}|$ in Equation (1) we find that $|\dot{x}| = 0.366$ cm./sec. According to Equation 72 (3) the output is $[dW/dt] = \frac{1}{2}\pi a^2 \rho_0 c |\dot{x}|^2 = 0.524$ watt, giving a transmission per unit area of 0.00102 watt/cm². According to Equation 79 (3) the axial intensity is given by

$$I(0) = \frac{e^{-2\kappa' r}}{r^2} \frac{(\kappa_r a)^2}{4\pi} \left[\frac{dW}{dt}\right] = 0.273 \times 10^{-9} \text{ erg/cm.}^2$$

On the decibel scale this coιιesponds to a reception level of 64.3 db.

These results, deduced from observation, may be compared with the numerical data employed in the writer's paper as shown in Table I.

TABLE I

CHARACTERISTICS OF PIEZO-ELECTRIC TRANSMITTERS

—	60-cm. oscillator (theory)	10-in. oscillator (estimated from observations)
Total output	50 watts	0.524 watt
Circumference/wave-length	50	53.9
Transmission per unit area	0.0177 watt/cm.²	0.00102 watt/cm.²*
Velocity amplitude of piston	1.53 cm./sec.	0.366 cm./sec.

Originally, in the absence of numerical data, the amplitude of the 50-watt oscillator was chosen to make the maximum pressure-amplitude at the centre of the disk less than atmospheric (or cavitation) pressure by a factor of safety of 4, according to Formula 40*(3).

In order to have a useful optimum chart, reception intensities require to be condensed by the use of a logarithmic scale. This is conveniently provided for by the decibel scale now in international use. It is also necessary to refer the chart to some standard diameter and output of the transmitter, such as 60 cm. and 50 watts, although these specifications and ranges implied are probably considerably greater than those prevailing in present practice. As it stands it provides an objective for designers of practical transmitters. It may, however, by the principle of similitude, be used for a transmitter of any given radius and output. For a given intensity of reception, let r' be the optimum range of a transmitter of radius a' and output $[dW'/dt]$ at optimum wave-length λ'. It follows from Equations 81 and 80 (3) that

$$\left(\frac{r'}{r}\right)^3 = \left(\frac{a'}{a}\right)^2 \frac{[dW'/dt]}{[dW/dt]} \quad \text{and} \quad \frac{\lambda'}{\lambda} = \left(\frac{r'}{r}\right)^{\frac{1}{2}}. \qquad (2)$$

To use these formulas in relation to the optimum reception chart of Fig. 2, we take $a = 30$ cm. and $[dW/dt] = 50$ watts, and determine r and λ corresponding to the desired reception level, interpolating between the 0° and 20° C. curves for the given sea temperature. The procedure will be made clear by finding the theoretical optimum range and wave-length for 64.3 db. reception

* *The wide divergence in the above figures for transmission per unit area shows the need for observations on the maximum output of an actual piezo-electric transmitter consistent with absence of cavitation (1).*

from the 10-in. transmitter of 0.524 watt output referred to in Table I. From the chart we find by interpolation that at 7° C., we have for $a = 30$ cm., $[dW/dt] = 50$ watts, $r = 34$ km., $\lambda = 4.0$ cm. Then, according to Formulas (2) above,

$$\left(\frac{r'}{34}\right)^3 = \left(\frac{12.8}{30}\right)^2 \frac{0.524}{50}, \text{ giving } r' = 4.09 \text{ km. and } \frac{\lambda'}{4.0} = \left(\frac{4.09}{34}\right)^{\frac{1}{2}}, \text{ or } \lambda' = 1.39 \text{ cm.}$$

corresponding to a frequency $f = 107{,}000$.

These results show from the observational data supplied by Dr. Boyle that the transmitter was operating close to theoretical optimum conditions.

The use of the decibel scale in estimating reception is practically convenient because many data are now available as to intensity levels susceptible of measurement and transmission in air. For instance a level of 64 db. is somewhat less than that of an average voice speaking into a telephone transmitter, and is thus known to possess sufficient energy to be susceptible of easy measurement.

The level of 30 db., assumed in the curves of Fig. 1, as a probable lower limit of detectable intensity was estimated by the writer from experience with sound-measuring instruments in air. It was found that a Webster phonometer used in measuring the strength of fog signals would give an easily audible, detectable reading of 0.1 mm. for a pressure variation $|\delta p| = 0.000204$ bar (2, p. 249). This corresponds to a level of 50 db. The disk of the instrument was purposely loaded to make it insensitive; it was estimated that by suitable adjustment its sensitivity could have been increased by a factor of 10, corresponding to a level of 30 db. It therefore appears probable to the writer that a piezo-electric receiver, designed to make best use of mechanical resonance, and utilizing high amplification, should be capable of detecting a reception level of 30 db. in water; in all events this should be the objective for the design of such a receiver. When the reception level of the receiver is known and the output and radius of the transmitter are given, a set of curves corresponding to those of Fig. 1 are easily computed from Formula 79 (3).

The important feature of an optimum transmitting frequency for a given range of transmission was first pointed out by Dr. Boyle in 1918, although arrived at independently by the writer as a theoretical consequence of the effect of viscosity on transmission. Quoting from a report of tests carried out in 1918 he states:

"The theoretical results brought out here, namely, that for any given distance there is a best frequency at which to transmit, and also that for long distances the lower frequencies will travel better than the higher ones (*i.e.*, will be less damped out by the viscosity) receive a good deal of experimental support from our experiments.

For work in the open sea over the greater part of the year, the temperature of the water will be higher than the temperature considered here, *viz.*, 7° C. The coefficient of viscosity therefore will be less than the value used here (0.017), and therefore the best frequencies will have values slightly higher

than those represented here; also, waves of any frequency ought to travel better in the summer than in the winter. Another fact to be taken into consideration is that in any practical supersonic system a part of the energy reaching the receiver is due to the supersonic waves reflected from the water surface. The path of such waves between transmitter and receiver must necessarily be larger than that of the direct waves, and on this account the frequency could with advantage be lowered a little.

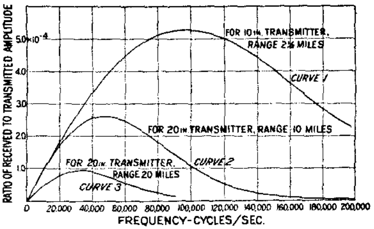

FIG. 1. *Calculated ratio of received to transmitted amplitude. (Courtesy of Dr. Boyle).*

In conclusion it must be stated that these calculations can be approximate only, since it is impossible to say how far the first assumption of paragraph 1* is justified for waves of such high frequencies as are used in supersonics. But the results seem to agree with the practical observations that waves of lower frequency travel better than those of very high frequency, and consequently in endeavoring to obtain ranges of five miles or upwards it will be necessary to work at frequencies of about 70,000 cycles per sec. and lower, unless large amounts of energy can be put into the water."

Figs. 1 and 2 show curves which Dr. Boyle has very kindly allowed the writer to include in this paper.

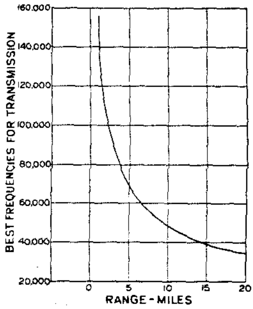

FIG. 2. *Optimum frequencies for transmission (calculated).*

To compare these results with the writer's formulas, we note from Equation (1) that, writing $|\dot{x}| = \omega |x|$ we have

$$\frac{|\xi|}{|x|} = \frac{1}{2} a^2 \kappa_0 \frac{e^{-\kappa' r}}{r} \quad . \tag{3}$$

It is readily proved that assuming $|x|$ constant as the frequency is varied, the ratio $|\xi|/|x|$ for a given r has a maximum for $\lambda = 4\pi \left(\frac{\mu r}{3 \rho_0 c} \right)^{\frac{1}{2}}$ as in Equation 80 (3). With $r = 4 \times 10^6$ cm., $\mu_7 = 0.016$, $\rho_0 c = 1.53 \times 10^5$, we find $\lambda = 1.48$ cm., corresponding to a frequency $f = 100{,}000$. With this value of λ we find

$$\frac{|\xi|}{|x|} = \frac{\pi a^2}{\lambda r \sqrt{e}} = 5.26 \times 10^{-4} \quad .$$

* *i.e., the use of the coefficient of viscosity obtained from steady flow measurements.*

It will be noticed that these values of f and $|\xi|/|x|$ agree as closely as could be desired with the maximum of the curves calculated by Dr. Boyle (Figs. 2 and 3) for the 10-in. transmitter with a range of $2\frac{1}{2}$ miles.

Dr. Boyle points out that in practice the best signals from a 10-in. transmitter at $2\frac{1}{2}$ miles come in over a range of frequencies from 50,000 to 80,000. A value of μ_7 double that assumed from capillary flow experiments would make the optimum frequency come out about right. This to some extent supports Biquard's contention alluded to in Section 12 (3), that the effective viscosity at supersonic frequencies is considerably greater than the values usually assumed.

On the other hand, Equation (3) presupposes the existence of an infinite rigid flange; it is possible that the appropriate formula for a flangeless transmitter would give an optimum frequency in better accord with observation with the usual value of viscosity.

The determination of the optimum wave-length from Equation (3) also assumes that the transmitter amplitude $|x|$ remains constant as the frequency is varied. Should $|x|$ be a function of the frequency, as might well be the case if the transmitter is operated near its natural resonance in spite of a constant applied voltage, the frequency corresponding to maximum displacement-amplitude $|\xi|$, or signal strength would be shifted to the right or left of that calculated for constant transmitter amplitude $|x|$ and the usual value of the coefficient of viscosity.

It is evident that accurate data on the coefficient of viscosity of sea water at supersonic frequencies are greatly needed in connection with the practical application of theory.

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EXPERIMENTS ON THE USE OF MIXTURES OF GASOLINE WITH ETHYL AND ISOPROPYL ALCOHOLS IN INTERNAL COMBUSTION ENGINES[1]

By M. S. Kuhring

Abstract

The paper covers engine tests conducted on gasoline and various mixtures of isopropyl and ethyl alcohols with gasoline. Comparisons were made with additions of lead tetraethyl in the gasoline. The tests were conducted in two separate groups and included octane number, power, consumption, and highest useful compression ratio.

It is shown that once detonation is suppressed in an engine, little or no further increase in power may be expected by raising the anti-knock value of the fuel, without also raising the compression ratio.

In the first group, specific fuel consumptions when using the ethyl alcohol–gasoline mixtures were higher than when gasoline only was used. This holds for fuels *A* and *C*. In the case of fuel *B* the specific consumption was found to be almost the same whether or not the alcohol was used.

When lead tetraethyl was added to an alcohol–gasoline mixture, there was a further increase in anti-knock value, but this increase was not as great as when the lead was added to the same gasoline without alcohol.

In group 2, specific fuel consumptions for gasoline–alcohol mixtures were higher than for gasoline only. In the 10% alcohol mixtures, consumptions were higher when ethyl alcohol was used than when isopropyl alcohol was mixed with the gasoline. In the 20% alcohol mixtures, the ethyl alcohol consumptions were still somewhat higher than those of the isopropyl mixtures; but with the 30% alcohol mixtures, specific consumptions were higher for the isopropyl alcohol additions than for ethyl alcohol additions.

Octane numbers, which were determined under C. F. R. Motor Method procedure, showed the ethyl alcohol to be superior as a knock inhibitor, but when the Highest Useful Compression Ratio was determined (under low cylinder temperature conditions) the 10% and 20% concentrations of isopropyl alcohol were found to be superior to ethyl alcohol in the same proportions. Above this concentration, ethyl alcohol became more effective than equivalent additions of isopropyl alcohol.

Range of Investigation

A report on the economic and technical feasibility of using as a motor fuel in Canada, gasoline mixed with alcohol made from grain, has been issued by the Division of Chemistry of the National Research Council (2). The experiments recorded in the present communication, which have been referred to briefly in the above report, deal with the effect on the operation of internal combustion engines of adding various proportions of ethyl and of isopropyl alcohol to representative samples of gasoline, and include a comparison of the relative effectiveness of additions of alcohol and lead tetraethyl on knocking. The effect of alcohol admixtures on the water tolerance of gasoline is the subject of a separate communication by Bayley and Hopkins (1). The work was conducted under two different sets of conditions, and the gasolines with which alcohol mixtures were made were not similar, consequently the two groups of tests will be treated separately.

[1] *Original manuscript received July 3, 1934.*

Contribution from the Engine Laboratory, Division of Physics and Engineering, National Research Laboratories, Ottawa, Canada.

Group I

Three samples of gasoline obtained for this work were as follows:

A. Straight run aviation gasoline, non-ethyl.

B. Cracked, casing-head blend, non-ethyl.

C. Similar to sample *B*.

No definite information was obtained as to the source or properties of these fuels, but they were commercial grades as sold on the open market. Test samples were made up by mixing these fuels with 99.75% ethyl alcohol (See Table I).

Methods and Apparatus

The anti-knock quality, or octane number, of the various fuels was determined on the Ethyl Gasoline Corporation Series 30B detonation engine. This equipment consists of a single-cylinder, liquid-cooled engine, coupled to a synchronous motor with a vee belt. Speed may be varied by changing the pulley on the synchronous motor. Cooling liquid temperature is governed by a fractionating column and condenser, and may be set at any point between 212° and 390° F. Change in compression ratio is accomplished by inserting shims between the cylinder base and the crank case. The compression pressure used for testing the various samples was changed when necessary to give the same knock intensity at full throttle. Knock intensity was indicated by the Bouncing Pin and Knock Meter.

The anti-knock quality of the samples was determined under two sets of conditions, that is, cooling liquid temperature 212° F. and engine speed 600 r.p.m.; and C.F.R. Motor Method (cooling liquid 345° F. and engine speed 900 r.p.m.). In both cases the intake air was at room temperature.

Power curves, throttle curves, consumption, and highest useful compression ratio (H.U.C.R.) were determined on the Armstrong Whitworth variable compression fuel research plant.

This apparatus consists of a single-cylinder, variable-compression, water-cooled engine coupled to an electrical swinging-field dynamometer. Fuel consumption was measured by means of a flow meter.

Gum tests were conducted by the copper dish method.

Octane Number

The octane numbers of the three gasolines and of their mixtures with alcohol were determined by the C.F.R. Research and C.F.R. Motor Methods on the Series 30B engine.*

The results of these tests are shown in Table I and Fig. 1. The solid lines show the octane numbers obtained by the C.F.R. Motor Method, the dotted lines those determined by the C.F.R. Research Method. The difference in octane number under the two sets of conditions becomes less as the proportion of alcohol is increased.

**C.F.R. (Co-operative Fuel Research) Research Method calls for a cooling liquid temperature of 212° F. and an engine speed of 600 r.p.m. For the Motor Method, when using the Series 30B engine, it is necessary to have a cooling liquid temperature of 345° F. and an engine speed of 900 r.p.m. with the air intake at room temperature.*

TABLE I

INCREASE IN OCTANE NUMBER AND H.U.C.R. WITH ALCOHOL ADDITION

Sample No.	Fuel and percentage by vol.		Ethyl alcohol % by vol.	Octane number		H.U.C.R.
				C.F.R. Research Method*	C.F.R. Motor Method**	
1	*A*,	100	0	68	63	6.10 : 1
2	*A*,	95	5	71	67.5	6.25 : 1
3	*A*,	90	10	75	72	6.40 : 1
4	*A*,	85	15	79	76.5	6.82 : 1
5	*B*,	100	0	67	61.5	5.90 : 1
6	*B*,	95	5	70	66	6.00 : 1
7	*B*,	90	10	74	70.5	6.10 : 1
8	*B*,	85	15	78.5	75	6.40 : 1
9	*C*,	100	0	65	61	5.83 : 1
10	*C*,	95	5	67.5	66.4	5.90 : 1
11	*C*,	90	10	71.5	70.4	6.00 : 1
12	*C*,	85	15	76.5	75	6.26 : 1

**Co-operative Fuel Research, Research Method for Series 30B engine, 212° F., 600 r.p.m.*
***Co-operative Fuel Research Motor Method for Series 30B engine, 345° F., 900 r.p.m.*

It may appear unusual for straight run gasoline to show a greater loss in octane number than the cracked gasoline *C*, but as noted above, no tests were conducted to show what proportion of unsaturated hydrocarbons or benzol was present in any of these fuels.

It might also be mentioned in passing, that while it is not customary to

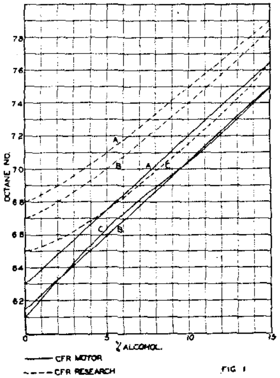


FIG. 1. *Increase in octane number with alcohol addition.*

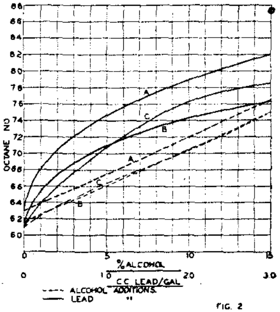


FIG. 2. *Increase in octane number with alcohol or lead tetraethyl addition.*

report knock ratings to less than one octane number, in these tests the readings have been reported to less than one octane number if the test so indicated.

There appears to be a slight irregularity in the Motor Method curve for Fuel *C* where it crosses and recrosses the Fuel *B* curve, but this point was carefully rechecked with no change in result. It would appear that in each of the three fuels the addition of alcohol produced much the same gain in anti-knock quality.

TABLE II

INCREASE IN OCTANE NUMBER WITH ADDITION OF LEAD TETRAETHYL

Lead addition cc. per gal.	Octane number		
	Fuel *A*	Fuel *B*	Fuel *C*
0.0	63	61.5	61
1.0	74.6	71	70.8
2.0	79	74.3	76.3
3.0	82	76.3	78.5

Comparison of the Effects of Alcohol and of Lead Tetraethyl Additions on Knock Rating

The fuels *A* (Sample 1), *B* (Sample 5), and *C* (Sample 9) were treated with lead tetraethyl in the proportions of 1.0, 2.0, and 3.0 cc. per gallon and the octane number determined (Table II).

These results, obtained under the C.F.R. Motor Method, are shown in Fig. 2, in which the vertical ordinates show *either* the percentage of alcohol addition *or* the number of cubic centimetres of lead tetraethyl per gallon.

The effect of lead tetraethyl addition in conjunction with alcohol has been examined in one case where 3.0 cc. of lead tetraethyl was added to the 85–15% mixture of Fuel *A* (Sample 4). (Table III.)

TABLE III

INCREASE IN OCTANE NUMBER OF FUEL *A* DUE TO ADDITION OF ALCOHOL AND/OR LEAD TETRAETHYL

Gasoline *A*, %	Alcohol, %	Lead tetraethyl, cc. per gal.	Octane number
100	—	—	63
85	15	—	76.5
100	—	3	82.0
85	15	3	87.3

This effect is also illustrated by the point in the upper right-hand corner of Fig. 2.

Highest Useful Compression Ratio (H.U.C.R.)

The H.U.C.R. for each of the 12 samples was determined on the Armstrong Whitworth engine. The engine was run at a compression ratio low enough to avoid detonation. The air-fuel ratio was set at the leanest mixture for maximum power. The compression ratio of the engine was then increased, the speed being kept constant, until the power reached a maximum and began to fall off. The air–fuel ratio was then checked and the compression ratio again tested. This compression ratio was taken as the Highest Useful Compression Ratio (H.U.C.R.). The engine was operated at a cooling temperature of 210° F. for both cylinder jacket and cylinder head, and at a speed of 750 r.p.m. (For results see Table I and Fig. 3.)

Power and Throttle Curves

Power, throttle and consumption curves were taken on the Armstrong Whitworth variable compression engine. For the power curves, the engine was operated at full throttle power at 750 r.p.m. and the fuel consumption and developed power noted. The engine was then braked down in 100 r.p.m. steps by means of the dynamometer. After constant temperature conditions had been attained, power and consumption readings were taken at each 100 r.p.m. interval. The throttle remained locked in its full open position during this test.

For throttle curves, the engine was operated at full throttle power, and set at 750 r.p.m. by means of the dynamometer. The dynamometer control was then locked and the engine speed governed by closing the throttle. Consumption figures were not taken for throttle curves.

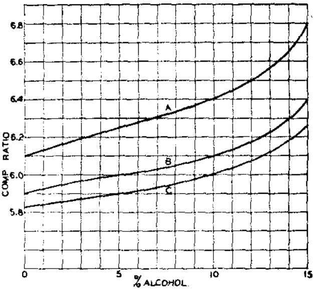


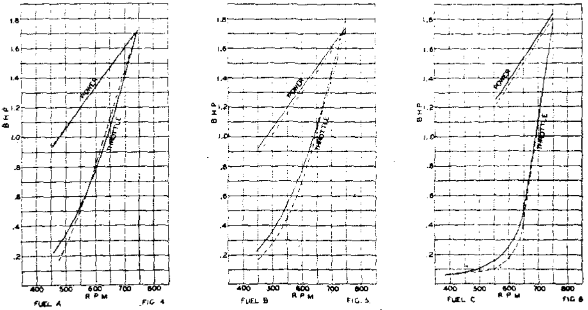
FIG. 3

FIG. 3. *Increase in H.U.C.R. with alcohol addition.*

Tests were conducted on:

Sample 1 (Fuel *A*).
Sample 4 (Fuel *A* 85%, alcohol 15%).
Sample 5 (Fuel *B*).
Sample 8 (Fuel *B* 85%, alcohol 15%).
Sample 9 (Fuel *C*).
Sample 12 (Fuel *C* 85%, alcohol 15%).

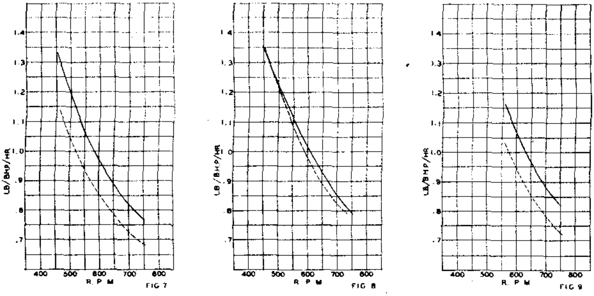
Figs. 4, 5 and 6 show power and throttle curves for the fuels and mixtures



FIGS. 4, 5 and 6. *Power curves of Fuels A, B and C, with and without alcohol.*

respectively, while Figs 7, 8 and 9 show specific consumptions for the power curves. Solid lines indicate alcohol–gasoline mixtures while broken lines indicate gasoline only. Power has been corrected to 60° F. as the air intake temperature was subject to quite wide variation. Fuel consumptions are given for power curves only and are expressed in lb./B.H.P./hr.

As it is not feasible to change the compression ratio of automobiles already in use, it was decided to use one compression ratio for power curves of all samples, and in this way to see what the effect of the various fuels would be under certain engine conditions. For this reason, the power obtained in some cases is not as high as it would have been had advantage been taken of the high anti-knock value of the fuel, to increase the compression ratio.



FIGS. 7, 8 and 9. *Specific fuel consumption curves for Fuels A, B and C and mixtures.*

A compression ratio of 6 : 1 was selected. This is not as high as that employed in some engines but is higher than the average. In addition, this compression ratio marks the point where Fuel *A* without alcohol (Sample 1) resists detonation, while Fuels *B* and *C*, without alcohol (Samples 5 and 9 respectively) detonate.

Fuel A. As stated before, this fuel was capable of resisting detonation under the above engine conditions, without any alcohol addition. From past experience in this laboratory,* it has been found that once detonation has been suppressed, little if any gain in power can be expected by increasing the anti-knock value of the fuel without also raising the compression ratio. This is strongly supported by the power curve in Fig. 4. The same is true to a large extent in the throttle curve, though there is a certain amount of difference due to the difficulty in setting the throttle in exactly the same place each time.

Consumption of the alcohol–gasoline mixture was definitely higher than of the gasoline alone. When considering these consumptions, it must be

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remembered that if full advantage had been taken of the possible increase in compression ratio, a probable gain in power of about 6% would have resulted.

Fuel B. Without alcohol this fuel was not capable of suppressing detonation at the 6 : 1 compression ratio, but with the 15% alcohol addition, this was overcome as is shown by the 1.2% increase in power at 750 r.p.m. and the gain of 3.4% at 450 r.p.m. As in the case of Fuel *A*, a slight increase in power might have been obtained by increasing the compression ratio for the alcohol–gasoline mixture.

The specific fuel consumption (Fig. 8) for the alcohol–gasoline mixture was practically the same as for the gasoline alone at 450 and at 750 r.p.m., while in the middle range it was 4–5% greater.

Fuel C. The gain in power of this fuel with 15% alcohol addition was 1.5% at 750 r.p.m. and 2.8% at 560 r.p.m. The curve was not taken to lower speeds, as a slight irregularity in the operation of the engine gave inaccurate results.

Specific fuel consumption increased for the alcohol–gasoline mixtures.

Gum

It was decided to subject the fuels, with their various blends to a copper dish "gum" test. The result of this test was as follows:

	Fuel *A*				Fuel *B*				Fuel *C*			
Sample number	1	2	3	4	5	6	7	8	9	10	11	12
"Gum" (mgm. per 100 cc.)	−2	−2	3	3	15	16	28	14	37	38	41	35

A certain amount of light corrosion was noted with the alcohol alone but no weighable amount of residue was found.

In all cases the gum content did increase slightly from the 100% gasoline to the 10% alcohol mixture, and dropped again with the 15% alcohol mixture. It is not thought that the alcohol had much effect on the gum, but it is interesting to note a similar rise and fall in each of the fuels tested.

The effect of storage was not considered, as the accelerated aging equipment has not yet been completed.

Ease-of-starting Test

Since much has been said about the difficulty of starting engines with fuels containing large quantities of alcohol, it was decided to try starting the engine on alcohol alone. The engine was run for a time on ethyl alcohol only, and then closed down for the night. The cooling water was allowed to circulate through the cylinder jacket, and the room temperature dropped to about 60° F. The next morning, with only alcohol in the fuel system, the engine was motored over at approximately 450 r.p.m. and began to fire in about 15 sec., showing little reluctance to starting. Several other starts were made in the same manner when the engine had been stopped for shorter periods.

Group 2

This section deals with tests which were similar to those in Group I, but in which the operating conditions were somewhat different.

Specific gravity at 60° F.	0.706	50% point	92.0° C.
Gum	0.4 mg. per 100 cc.	60% point	97.5° C.
Corrosive sulphur	Nil	70% point	103.5° C.
Total sulphur	0.002% by weight	80% point	111.0° C.
Initial boiling point	50° C.	90% point	122.5° C.
10% point	68° C.	End point	160.0° C.
20% point	74.5° C.	Recovery	99.0%
30% point	81.0° C.	Residue	0.9%
40% point	86.5° C.		
		Loss	00.1%

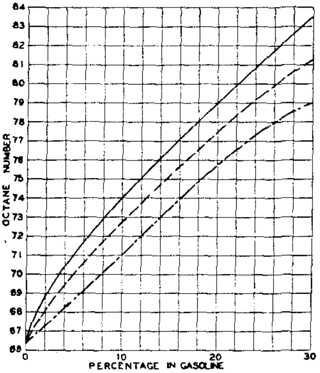


FIG. 10

FIG. 10. *Octane number of gasoline with and without additions of ethyl and/or isopropyl alcohol.* ——— *Ethyl alcohol;* – – – – *ethyl and isopropyl alcohols;* —·—·— *isopropyl alcohol.*

The gasoline which was used for a base fuel was a straight run, non-ethyl gasoline, with the above characteristics.

With this fuel were mixed various proportions of ethyl and/or isopropyl alcohol (See Table IV). The ethyl alcohol was 99.75% pure (by volume). The isopropyl alcohol contained 2.38%, by volume, of water.

Octane Number

The octane numbers were determined on the Series 30B engine under the C.F.R. Motor Method as described in the previous section, for gasoline alone, and for each of the alcohol–gasoline mixtures. The results are shown in Table IV and in Fig. 10.

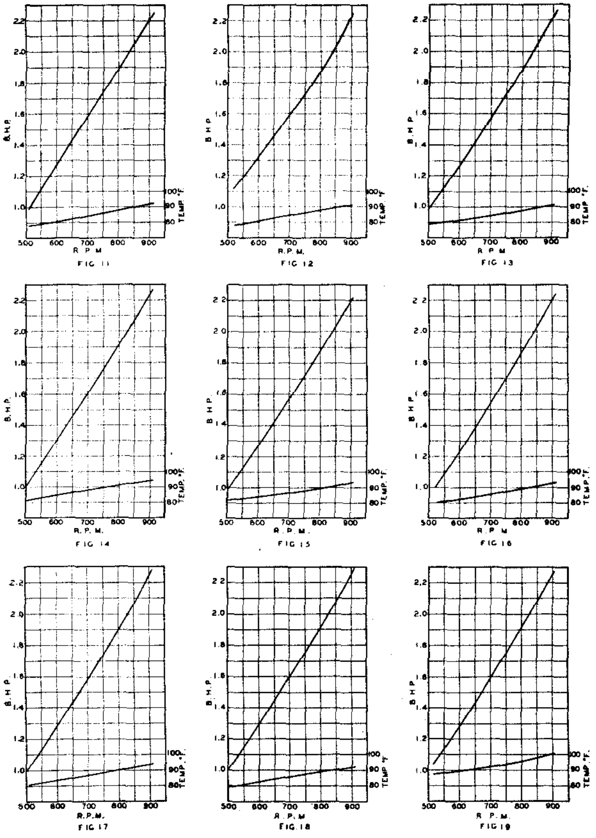
TABLE IV

INCREASE IN OCTANE NUMBER DUE TO ALCOHOL ADDITIONS

Sample No.	Gasoline, %	Ethyl alcohol, %	Isopropyl alcohol, %	Octane number C.F.R. Motor Method
13	100	0	0	66.4
14	90	10	0	74.0
15	80	20	0	79.0
16	70	30	0	83.5
17	90	5	5	72.8
18	80	10	10	77.5
19	70	15	15	80.3
20	90	0	10	71.0
21	80	0	20	75.75
22	70	0	30	79.0

Power and Consumption Curves

Power and consumption curves were found on the Armstrong Whitworth variable-compression engine.



FIGS. 11–19. *Power curves for gasoline only, gasoline-ethyl alcohol, gasoline-ethyl alcohol-isopropyl alcohol, and gasoline-isopropyl alcohol. Fig. 11 corresponds to Table V, Fig. 12 to Table VI, and so on to Fig. 19 which corresponds to Table XIV.*

The engine was thoroughly warmed and set at a compression ratio of 5.5 : 1 to permit the use of gasoline without detonation. The throttle was fully opened and the fuel mixture set as lean as possible without loss of power.

After running at 900 r.p.m. for three minutes, readings were taken. With the throttle in the full open position, the engine was slowed to 700 r.p.m. by means of the electrical dynamometer. Readings were taken again at the end of three minutes. This was repeated down to 500 r.p.m. and back again to 900 r.p.m. The same procedure was carried out for each blend and the power developed is shown in Figs. 11–20 and in Tables V–XIV.

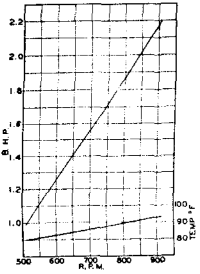


FIG. 20

FIG. 20. *Power curves for gasoline and isopropyl alcohol. This figure corresponds to Table XIV.*

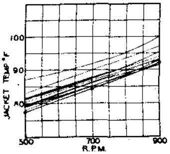


FIG 21

FIG. 21. *Temperature of the cooling water at the outlet during determination of power curves.*

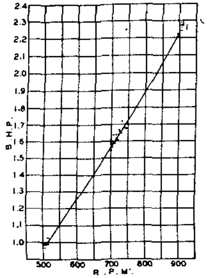


FIG. 22

FIG. 22. *Composite power curve—Fuels 13–22.*

The temperatures of the cylinder jacket cooling liquid are given. The cylinder head cooling, which is separate, was kept constant at 212° F.

Fig. 21 gives the temperatures of the cooling water leaving the engine during all of the power tests, the curve marked with circles being that of the gasoline alone. There is variation between curves due mainly to irregularity in temperature of the inlet water which could not be controlled, but all the curves are very similar in slope.

TABLE V

RESULTS OBTAINED WITH 100% GASOLINE

R.p.m. (actual)	*915*	*920*	*710*	*710*	*500*	*510*	*720*	*720*	*905*	*900*
Wt. supported, lb.	8.55	8.55	7.80	7.82	6.70	6.80	8.0	8.0	8.75	8.675
B.H.P. (observed)	2.24	2.25	1.58	1.59	.96	.99	1.64	1.64	2.26	2.23
Fuel, pt./hr.	1.78	1.78	1.70	1.70	1.60	1.62	1.70	1.70	1.77	1.76
Fuel, lb./B.H.P./hr.	.705	.702	.96	.95	1.48	1.46	.92	.92	.70	.70
Cyl. jacket, ° F.	95	94	88	87	83	78	81	84	88	91
Air inlet, ° F.	78	78	78	78	78	78	78	77.5	77.5	77.5
Pressure drop across $\frac{1}{3}$-in. orifice on meter, in. of water	1.94	1.94	1.13	1.13	.56	.63	1.25	1.25	1.94	1.94

Pressure 760.4 mm.

TABLE VI

RESULTS OBTAINED WITH GASOLINE 90%, ETHYL ALCOHOL 10%

R.p.m. (actual)	910	905	710	712	505	520	720	725	900	900
Wt. supported, lb.	8.70	8.65	7.88	7.90	6.70	6.90	8.05	8.03	8.75	8.80
B.H.P. (observed)	2.26	2.24	1.60	1.61	0.97	1.03	1.66	1.66	2.25	2.26
Fuel, pt./hr.	1.97	1.97	1.88	1.88	1.78	1.78	1.87	1.85	1.91	1.92
Fuel, lb./B.H.P./hr.	.78	.79	1.06	1.05	1.65	1.56	1.01	1.00	.76	.76
Cyl. jacket, ° F.	92	92	88.5	86	82	78	83	84.5	88	91
Air inlet, ° F.	78	78	78	78	78	77.5	77.5	77.5	77.5	77.5
Pressure drop across ½-in. orifice on meter, in. of water.	1.94	1.94	1.13	1.16	.59	.59	1.19	1.19	1.94	1.94

Pressure 760.4 mm.

TABLE VII

RESULTS OBTAINED WITH GASOLINE 80%, ETHYL ALCOHOL 20%

R.p.m. (actual)	910	900	705	705	500	500	720	720	905	910
Wt. supported, lb.	8.65	8.70	7.88	7.90	7.1	6.9	8.00	7.95	8.65	8.75
B.H.P. (observed)	2.25	2.24	1.59	1.59	1.01	0.99	1.65	1.64	2.24	2.28
Fuel, pt./hr.	1.98	1.97	1.89	1.88	1.78	1.77	1.86	1.86	1.92	1.92
Fuel, lb./B.H.P./hr.	.80	.80	1.08	1.07	1.60	1.63	1.03	1.03	.78	.76
Cyl. jacket, ° F....	92	92.5	90	85	84	79	80.5	85	87	91
Air inlet, ° F.	78	78	77.5	77.5	77	77	77	77	77	77
Pressure drop across ½-in. orifice on meter, in. of water.	1.91	1.91	1.09	1.13	.56	.56	1.19	1.19	1.91	1.91

Pressure 760.4 mm.

TABLE VIII

RESULTS OBTAINED WITH GASOLINE 70%, ETHYL ALCOHOL 30%

R.p.m. (actual)	920	910	705	700	505	500	710	714	910	910
Wt. supported, lb.	8.80	8.75	8.0	8.05	7.15	7.0	8.05	8.05	8.6	8.75
B.H.P. (observed)	2.31	2.28	1.61	1.61	1.03	1.00	1.63	1.64	2.24	2.28
Fuel, pt./hr.	2.04	2.02	1.90	1.90	1.80	1.76	1.84	1.84	1.96	1.94
Fuel, lb./B.H.P./hr.	.81	.82	1.08	1.08	1.61	1.62	1.04	1.03	.80	.78
Cyl. jacket, ° F.	96	96	95	89	88	81.5	84	88	88	93
Air inlet, ° F.	80	80	79.5	79.5	79.5	79	79	79	79	79
Pressure drop across ½-in. orifice on meter, in. of water	2	2	1.06	1.09	.56	.56	1.13	1.09	2	2

Pressure 771 mm.

TABLE IX

RESULTS OBTAINED WITH GASOLINE 90%, ETHYL ALCOHOL 5%, ISOPROPYL ALCOHOL 5%

R.p.m. (actual)	900	915	710	702	505	503	720	725	900	895
Wt. supported, lb.	8.5	8.5	7.9	7.85	6.9	6.9	7.9	8.0	8.7	8.7
B.H.P. (observed)	2.19	2.22	1.60	1.57	1.00	.99	1.63	1.66	2.24	2.22
Fuel, pt./hr.	1.83	1.84	1.73	1.74	1.62	1.62	1.72	1.72	1.80	1.79
Fuel, lb./B.H.P./hr.	.75	.75	.97	1.00	1.45	1.47	.95	.93	.72	.73
Cyl. Jacket, ° F.	94	94	93	88	85	81.5	81	87	89	92
Air inlet, ° F.	78	78	78	78.5	78.5	78.5	79	79	79	79.5
Pressure drop across ½-in. orifice on meter, in. of water	1.94	1.97	1.13	1.13	.44	.44	1.25	1.22	1.94	1.91

Pressure 771 mm.

TABLE X

RESULTS OBTAINED WITH GASOLINE 80%, ETHYL ALCOHOL 10%, ISOPROPYL ALCOHOL 10%

R.p.m. (actual)	910	911	690	690	515	515	730	740	920	920
Wt. supported, lb.	8.6	8.6	7.6	7.6	6.8	6.8	7.7	7.95	8.55	8.65
B.H.P. (observed)	2.24	2.24	1.50	1.50	1.00	1.00	1.61	1.68	2.25	2.27
Fuel, pt./hr.	1.96	1.95	1.85	1.84	1.76	1.75	1.85	1.83	1.95	1.95
Fuel, lb./B.H.P./hr.	.80	.79	1.12	1.12	1.60	1.59	1.04	.99	.79	.78
Cyl. jacket, ° F.	95	95	93	86.5	85	80	79	85	87	92
Air inlet, ° F.	78	78	78	78	78	78	78	78	78	78
Pressure drop across ½-in. orifice on meter, in. of water	2	2	1.06	1.06	.59	.63	1.28	1.31	2	2.03

Pressure 771 mm.

TABLE XI

RESULTS OBTAINED WITH GASOLINE 70%, ETHYL ALCOHOL 15%, ISOPROPYL ALCOHOL 15%

R.p.m. (actual)	915	905	704	704	505	503	702	703	910	910
Wt. supported, lb.	8.80	8.75	7.9	7.9	6.95	6.95	7.85	7.9	8.7	8.8
B.H.P. (observed)	2.30	2.26	1.59	1.59	1.00	1.00	1.57	1.59	2.26	2.29
Fuel, pt./hr.	1.97	1.97	1.88	1.88	1.78	1.77	1.83	1.84	1.97	1.97
Fuel, lb./B.H.P./hr.	.79	.80	1.08	1.08	1.64	1.63	1.08	1.07	.80	.79
Cyl. jacket, ° F.	94	94	91.5	87	86.5	80.5	81	87	87	94
Air inlet, ° F.	78	78	78	78	78	78	78	78	78	78
Pressure drop across ½-in. orifice on meter, in. of water	2.03	2.03	1.13	1.13	.56	.56	1.16	1.16	1.97	2

Pressure 771 mm.

TABLE XII

RESULTS OBTAINED WITH GASOLINE 90%, ISOPROPYL ALCOHOL 10%

R.p.m. (actual)	910	915	703	700	495	500	720	730	910	905
Wt. supported, lb.	8.75	8.80	7.95	7.93	6.90	7.0	8.20	8.25	8.90	8.85
B.H.P. (observed)	2.28	2.30	1.60	1.59	.98	1.00	1.69	1.72	2.31	2.29
Fuel, pt./hr.	1.92	1.92	1.81	1.78	1.70	1.68	1.78	1.78	1.85	1.85
Fuel, lb./B.H.P./hr.	.756	.75	1.01	1.00	1.56	1.51	.94	.92	.72	.73
Cyl. jacket, ° F.	94	92	90	87	84	79	82	86	88	92
Air inlet, ° F.	78.5	78.5	78.5	78	78	78	78	78	78	78
Pressure drop across ½-in. orifice on meter, in. of water	2	2	1.13	1.13	.56	.56	1.25	1.25	2	2

Pressure 771 mm.

TABLE XIII

RESULTS OBTAINED WITH GASOLINE 80%, ISOPROPYL ALCOHOL 20%

R.p.m. (actual)	900	905	703	704	505	513	720	723	910	900
Wt. supported, lb.	8.75	8.80	7.90	7.90	6.95	7.05	8.08	8.10	8.80	8.83
B.H.P. (observed)	2.25	2.28	1.59	1.59	1.00	1.03	1.66	1.67	2.29	2.27
Fuel, pt./hr.	1.98	1.97	1.85	1.85	1.75	1.75	1.85	1.85	1.94	1.92
Fuel, lb./B.H.P./hr.	.80	.78	1.05	1.05	1.59	1.55	1.01	1.01	.77	.77
Cyl. jacket, ° F.	111	111	105	98	94	87	91	91	92.5	97
Air inlet, ° F.	77.5	78	78	77.5	77.5	77.5	77.5	77.5	77.5	77.5
Pressure drop across ½-in. orifice on meter, in. of water	1.94	1.94	1.13	1.13	.56	.59	1.22	1.19	1.97	1.94

Pressure 770 mm.

TABLE XIV

RESULTS OBTAINED WITH GASOLINE 70%, ISOPROPYL ALCOHOL 30%

R.p.m. (actual)	900	900	700	700	505	510	715	720	900	900
Wt. supported, lb.	8.60	8.60	7.85	7.75	6.90	6.80	7.75	7.85	8.50	8.58
B.H.P. (observed)	2.21	2.21	1.57	1.55	1.00	.99	1.58	1.61	2.19	2.21
Fuel, pt./hr.	2.07	2.06	1.98	1.98	1.87	1.86	1.95	1.97	2.04	2.02
Fuel, lb./B.H.P./hr.	.86	.85	1.16	1.18	1.72	1.73	1.13	1.12	.85	.84
Cyl. jacket, ° F.	94	94	92	87.5	86	80	82	85	87.5	93
Air inlet, ° F.	77.5	77.5	77.5	77.5	77.5	77.5	77.5	77.5	77.5	77.5
Pressure drop across ½-in. orifice on meter, in. of water	1.91	1.91	1.06	1.06	.56	.56	1.13	1.13	1.91	1.91

Pressure 771 mm.

Fig. 22 is a composite power curve for all the samples and shows how little variation there is over the whole range. Figs. 23, 24 and 25 show the *specific fuel consumption in* lb./B.H.P./hr. For these consumptions, the specific gravities of the fuels were: gasoline 0.706, ethyl alcohol 0.789, isopropyl alcohol 0.789.

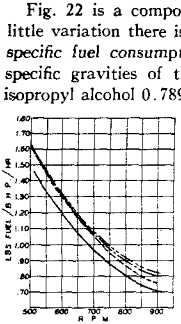


FIG. 23

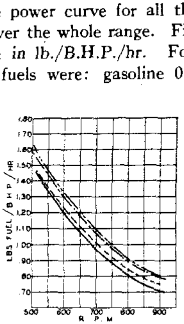


FIG. 24

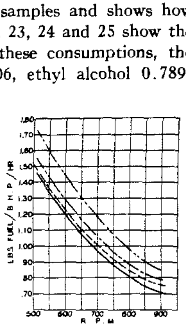


FIG. 25

FIGS. 23, 24 and 25. *Specific fuel consumption for power curves obtained with Fuels 13–22. Fig. 23.* ——— *100% Gasoline;* – – – – *90% gasoline, 10% ethyl alcohol;* — - — - *80% gasoline, 20% ethyl alcohol;* — – – — *70% gasoline, 30% ethyl alcohol. Fig. 24.* ——— *100% Gasoline;* – – – – *90% gasoline, 5% ethyl alcohol, 5% isopropyl alcohol;* — - — - *80% gasoline, 10% ethyl alcohol, 10% isopropyl alcohol;* — – – — *70% gasoline, 15% ethyl alcohol, 15% isopropyl alcohol. Fig 25.* ——— *100% Gasoline;* – – – – *90% gasoline, 10% isopropyl alcohol;* — - — - *80% gasoline, 20% isopropyl alcohol;* — – – — *70% gasoline, 30% isopropyl alcohol.*

Highest Useful Compression Ratio (H.U.C.R.)

Tests were conducted on the Armstrong Whitworth variable-compression engine. It was intended to run accurate H.U.C.R. tests on all mixtures of fuel in this section, but when the fuel which remained was examined, it was found that there was not sufficient left to carry out the procedure as outlined in the H.U.C.R. tests in Group 1 and still attain stabilized engine conditions. It was decided however, to run these tests with a modified procedure as far as the quantity of fuel would permit, and with these explanations, the information in this section is offered for what it may be worth.

The engine was thoroughly warmed on gasoline and the H.U.C.R. for this fuel found under the following conditions:

Speed, 900 r.p.m.; full throttle power; spark advance, 12°; cylinder head cooling, 212° F.; cylinder wall cooling, 120°F.; leanest mixture for maximum power.

The variable-compression plug was screwed down, increasing the compression ratio, until the power developed passed a maximum and began to drop. This compression ratio was taken as the H.U.C.R. and was the point taken for starting the tests with the various mixtures of alcohol and gasoline. Fig. 26 and Table XV show the results of this test on the different fuels.

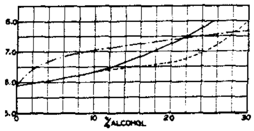


FIG. 26

• FIG. 26. *H.U.C.R. for Fuels 13–22.* ——— *Ethyl alcohol;* — - — - *isopropyl alcohol;* – – – – *ethyl and isopropyl alcohols.*

In addition to the H.U.C.R., the maximum power developed was noted, and these values are shown in Fig. 27. This power was the maximum power developed during the H.U.C.R. test and is incidental, as no attempt was made to attain maximum power other than by increasing the compression ratio.

TABLE XV

Gasoline, %	Ethyl alcohol, %	Isopropyl alcohol, %	Fuel No.	r.p.m.	Wt. lifted	B.H.P. obs. at actual r.p.m.	B.H.P. obs. at 900 r.p.m.	Pt./hr.	Fuel lb./B.H.P./hr.	Jacket temp., ° F.	H.U.C.R.
100	0	0	13	920	8.50	2.23	2.175	1.81	.728	121	5.9 : 1
90	10	0	14	920	8.7	2.29	2.22	1.97	.772	117	6.3 : 1
80	20	0	15	950	9.0	2.44	2.26	2.12	.791	115	7.25 : 1
70	30	0	16	950	9.2	2.50	2.315	2.19	.809	116	>8.0 : 1
90	5	5	17	930	8.65	2.30	2.195	2.01	.781	115	6.33 : 1
80	10	10	18	940	8.8	2.36	2.225	2.09	.809	119	6.62 : 1
70	15	15	19	955	9.2	2.51	2.305	2.15	.790	120	8.0 : 1
90	0	10	20	930	8.8	2.34	2.235	1.91	.736	119	7.05 : 1
80	0	20	21	945	8.9	2.40	2.245	2.05	.782	120	7.37 : 1
70	0	30	22	950	9.1	2.47	2.29	2.19	.818	120	7.65 : 1

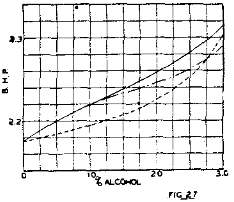


FIG. 27. *Maximum power noted during H.U.C.R. tests of Fuels 13–22.* ——— *Ethyl alcohol;* — — — *ethyl and isopropyl alcohols;* – – – – *isopropyl alcohol.*

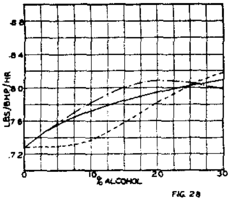


FIG. 28. *Specific fuel consumption noted during H.U.C.R. tests of Fuels 13–22.* ——— *Ethyl alcohol;* — — — *ethyl and isopropyl alcohols;* – – – – *isopropyl alcohol.*

Normally, the test would be run with the engine speed constant at 900 r.p.m., but as the maximum rise in speed of 55 r.p.m. as the compression ratio was increased would affect the results but little, and as the fuel was limited, it was necessary to permit this rise in speed to occur. Specific fuel consumptions taken during this test are shown in Fig. 28.

Conclusions

In tests by the C.F.R. Motor Method the increase in anti-knock value with the addition of ethyl alcohol is greater than the increase due to the addition of isopropyl alcohol. Alcohol additions composed of one half ethyl alcohol

and the other half isopropyl alcohol, produced an increase in anti-knocl value midway between those of additions consisting of the alcohols alone

As the power-consumption tests were run under conditions which did no permit detonation, there was very little variation in the power curves. A composite power curve (Fig. 22) shows the power curve for gasoline withou any alcohol (Sample No. 13) at the 500, 700 and 900 r.p.m. speeds. The power obtained for Samples 14–22 is shown by points. It will be noted that there is very little spreading of these points, which are within the accuracy of observation.

Temperatures in the cylinder jacket were fairly constant. While there was some slight variation, as shown in Fig. 21, this is largely due to temperature and pressure variation in the water supply, which could not be controlled.

Specific fuel consumptions were generally higher for alcohol–gasoline mixtures than for gasoline only. In the 10% alcohol mixtures, consumptions were higher when ethyl alcohol was used than when isopropyl alcohol was added. In the 20% mixtures the ethyl alcohol mixture consumptions were still somewhat higher than those of the isopropyl mixtures; but in the 30% mixtures, specific consumptions were higher for the isopropyl mixtures than for the ethyl alcohol mixtures.

In specific consumption, mixtures containing ethyl alcohol, isopropyl alcohol and gasoline fell between the mixtures containing either of the alcohols singly in the 30% mixture group. In mixtures with less than 30% alcohols the ethyl-isopropyl-gasoline has the lowest consumption.

Consumptions are given in lb./B.H.P./hr.

References

1. Bayley, C. H. and Hopkins, C. Y. Can. J. Research, 11 : 505—519. 1934.
2. Hopkins, C. Y. Preliminary report on the manufacture of grain alcohol for use in motor fuel. National Research Council Report. 1933.

WATER TOLERANCE OF MIXTURES OF GASOLINE WITH ETHYL ALCOHOL, ISOPROPYL ALCOHOL AND BENZENE[1]

By Colin H. Bayley[2] and Clarence Yardley Hopkins[3]

Abstract

The relation between water content and critical solution temperature of mixtures of gasoline with ethanol and isopropanol and with ethanol and benzene has been determined. Curves are presented which show the critical water contents of a wide range of mixtures at any temperature between +20° and −50° C. Three gasolines were used, two being straight-run and one a cracked gasoline. The mixtures contained 60 to 90% of gasoline with varying proportions of the other two components. Isopropanol has been found to bring about a marked increase in the critical water content of gasoline-ethanol mixtures to which it is added. Benzene is shown to be of little value for this purpose within the range of mixtures studied.

Introduction

The practice of using supplementary motor fuels in admixture with gasoline has increased steadily during the past few years. This trend is apparent chiefly in Europe among those countries which desire to be less dependent upon foreign sources of motor fuel. The gradual exhaustion of the world's petroleum deposits, which must be considered as inevitable, may be expected to result in the greater use of alternatives or substitutes for gasoline as it is now obtainable.

Owing to their availability, benzol and ethanol (ethyl alcohol) are in extensive use in the preparation of mixed motor fuels in which gasoline is the main constituent. Recent developments make it appear that isopropanol (isopropyl alcohol) can be manufactured cheaply from waste gases which are by-products of the petroleum industry, and it is not unlikely that in the near future this substance may become commercially available as a fuel.

A problem arises in connection with the use of mixed fuels containing ethanol, namely, the possibility of separation into two liquid phases upon the addition of relatively small amounts of water. At a given temperature any alcohol-gasoline mixture has a critical water content and conversely, any such mixture containing dissolved water has a critical solution temperature or "cloud" temperature below which separation into two phases will occur. This is of special practical importance in considering the feasibility of using blended fuels in countries in which low temperatures are encountered in winter.

Ternary mixtures of gasoline, ethanol and isopropanol were studied by Rothen and Boutier (7). Isopropanol was added to various mixtures of

[1] *Manuscript received June 14, 1934.*
Contribution from the Division of Chemistry, National Research Laboratories, Ottawa, Canada.

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gasoline with 96% ethanol until homogeneous solutions were obtained.* The range of critical solution temperatures investigated was +16° to −18° C. Whilst Rothen and Boutier do not describe the properties of the isopropanol, their results indicate that it contained several per cent of water.

Rothen and Boutier also examined mixtures in which the third component was benzol, but found that it was of little value in lowering the critical solution temperature.

Ternary mixtures of gasoline, benzol and ethanol have been studied by Legrand (5) who determined the critical solution temperatures of mixtures containing denatured 95% alcohol. A number of gasoline-ethanol-benzol mixtures were examined by Spausta (8) who determined the water tolerance at 20° C.

The cloud temperatures of a few mixtures of gasoline, ethanol and benzol with small amounts of water were determined by Swietoslawski, Pfanhauser and Bakowski (9). The critical solution temperatures were found to range from −22.5° C. to −75° C.

Baron, Boulanger and LeGrain (1) determined the cloud temperatures of a series of twelve mixtures of aviation gasoline, "motor benzole" and 99% ethanol.

King and Manning (4) investigated ternary mixtures containing benzol as the third component, the temperature range studied being −10° to +20° C. The limits of miscibility at these temperatures for mixtures prepared from alcohols of 94 to 98% strength (by volume) are shown on ternary diagrams.

Ormandy, Pond and Davies (6) determined the water tolerance of a long series of mixtures of gasoline and aliphatic alcohols at one temperature, *viz.*, 15° C.

The present investigation deals with the determination of the critical solution temperatures of mixtures of *(a)* gasoline and isopropanol, and *(b)* gasoline and benzene with ethanol containing known amounts of water. From these results, curves are constructed showing the critical water content for a wide range of ternary mixtures of the above substances.

Materials

Gasoline

The properties of the three gasolines used in this investigation are given in Table I. Gasolines *A* and *B* were commercial aviation gasolines, gasoline *C* was a regular grade automobile fuel. None of these gasolines contained tetraethyl lead.

Ethanol

A series of solutions of ethanol and water were prepared from commercial absolute alcohol. The water content of each solution was checked by density determinations. The data are given in Table II.

**i.e., various mixtures of gasoline with ethanol, the ethanol containing 96% of ethyl alcohol.*

TABLE I

PROPERTIES OF GASOLINES USED

	A	*B*	*C*
Type	Straight run aviation	Straight run aviation	Liquid phase cracked
Density $d^{15.6}_{15.6}$	0.738	0.715	0.728
Gum (mg./100 cc.)	0.4	0.4	16.0
Sulphur %	0.002	0.002	0.003
A.S.T.M. Distillation			
I.B.P. °C.	72	26	35
10%	91	68	55
20	96	74.5	69.5
30	101	81	84
40	106	86.5	99
50	110	92	112
60	115	97.5	124.5
70	120	103.5	138.5
80	127.5	111	155
90	139.5	122.5	163
End point	171.5	160	196
Recovery %	98	99	96
Residue %	1.3	0.9	1.6
Loss %	0.7	0·1	2.4

TABLE II

WATER CONTENT OF ETHANOL USED

No.	Alcohol, % by weight	Water, % by vol.*	No.	Alcohol, % by weight	Water, % by vol.*
1	93.03	5.65	6	96.62	2.69
2	93.99	4.85	7	96.71	2.62
3	95.08	3.97	8	97.18	2.25
4	96.00	3.20	9	97.78	1.69
5	96.23	3.01	10	97.96	1.63

* *Temperature, 15.6° C.*

Isopropanol

Two grades of isopropanol obtained respectively from the Eastman Kodak Company and from Schuchardt were used. Their properties are given in Table III.

TABLE III

PROPERTIES OF ISOPROPANOL USED

Alcohol	d^{20}_4	B.p. at 760 mm. Hg.	% water by vol.	d^{20}_4 after dehydration	B.p. at 760 mm. Hg. after dehydration, °C.
A (E.K.)	0.7927	80.4	2.38	0.7854	82.4
B (S)	0.7865	81.8	0.80	0.7855	82.4

Since the constants for the dehydrated alcohols are those of pure isopropanol, it follows that water was the only impurity present in the original materials. The identity of the dehydrated products was further shown by the fact that mixtures of gasoline and ethanol with the same volume of either of the above alcohols showed critical solution temperatures which did not vary by more than 1° C. The same was true if the alcohols were used before dehydration, provided that their respective water contents were taken into account when computing the total water content of the gasoline-ethanol-isopropanol mixture.

Benzene

Schuchardt's benzene "for molecular weight determination" was used. Since it showed no turbidity at −50° C., the water content may be taken as less than 0.01%, according to the extrapolated data of Groschuff (3). This quantity of water may be neglected for the purposes of the present investigation.

Experimental

The apparatus used consisted of a 6 × $\frac{5}{8}$-in. test tube fitted with a stopper carrying a thermometer and stirrer. This tube was supported in a larger tube which served as an air-chamber, the assembly being immersed in a mixture of acetone and carbon dioxide snow contained in a Dewar flask.

In making an experiment, about 10 cc. of the mixture was placed in the inner tube and cooled with continual stirring until the cloud point was reached. The assembly was then removed from the Dewar while the temperature of the mixture rose slowly, the stirring being continued. The point at which the cloud suddenly disappeared was taken as the critical solution temperature. The results of several determinations showed that the temperatures of appearance and disappearance of the cloud were identical. Repeated determinations with the same mixture gave identical readings for the cloud temperature, showing that there was no absorption of moisture by the mixture under the conditions of the experiment.

The calculation of the critical water contents has been based on the sum of the volumes of the components before mixing. This method neglects the change in volume on mixing gasoline and ethanol, but this change is sufficiently small to be neglected in the present experiments.

With mixtures containing gasoline, ethanol and benzene, the cloud point is more difficult to detect than when benzene is absent, and becomes still more obscure as the ratio of benzene to alcohol is increased. In the latter case, the cloud is very faint and breaks up into tiny droplets when the solution is cooled a few degrees below the cloud temperature. The cloud points of mixtures made with cracked gasoline were more difficult to determine than those containing straight run gasoline. The initial cloud in the former is little more than a bluish opalescence. This is especially true of mixtures in which the content of alcohol is less than 10%.

Frequent check determinations made with freshly prepared mixtures gave results agreeing to within 0.5° C. Temperatures were read to 0.5° C. and are recorded to the nearest degree. On account of the variable composition of gasoline it is to be expected that no two gasolines when mixed with equal volumes of alcohol would give mixtures having exactly the same critical solution temperatures. The results of Bridgeman and Querfeld (2) show that two gasolines having almost identical densities and distillation ranges gave mixtures whose cloud points differed by more than 2° C. For this reason it was felt that nothing was to be gained by recording temperatures to a fraction of a degree.

A. Gasoline-Ethanol

The critical solution temperatures of mixtures of gasolines *A* and *C* with ethanol containing various percentages of water are shown in Figs. 1 and 2.

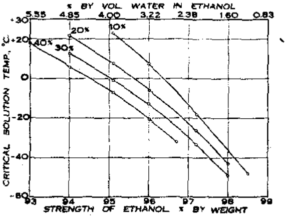

FIG. 1. *Critical solution temperatures of mixtures of gasoline C with ethanols of various strengths.*

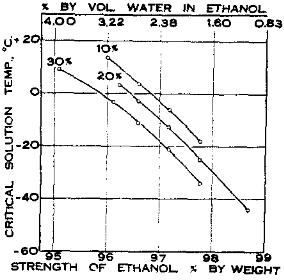

FIG. 2. *Critical solution temperatures of mixtures of gasoline A with ethanols of various strengths.*

B. Gasoline-Ethanol-Isopropanol

The critical solution temperatures of mixtures of gasoline, ethanol and isopropanol *A* containing 90% by volume of gasoline *A* are given in Fig. 3.

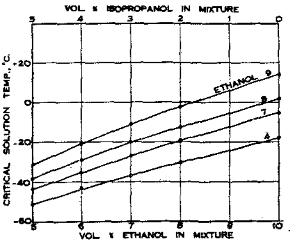

FIG. 3. *Critical solution temperatures of mixtures containing 90% gasoline A*

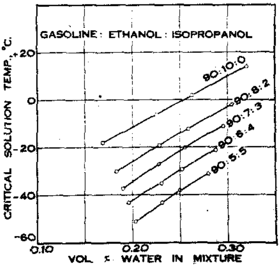

FIG. 4. *Critical water contents of mixtures containing 90% gasoline A.*

Four ethanol-water mixtures containing progressively less water were used in this series of experiments. The effect of water content is shown more clearly in Fig. 4 in which the water content, expressed as a percentage of the total mixture is plotted against critical solution temperature for various mixtures containing 90% of gasoline together with various proportions of ethanol and isopropanol.

It will be seen that for a given total water content of the mixture there is a slight though regular increase in the extent to which the critical solution temperature is lowered when the ethyl alcohol in the mixture is progressively replaced by isopropanol.

The increase in the critical water content of the mixture occasioned by increasing the percentage of isopropanol at the expense of the ethanol is shown in Table IV, in which the critical water contents of the first four of the above mixtures are compared for temperatures of 0°, −20° and −40° C.

TABLE IV

CRITICAL WATER CONTENTS OF MIXTURES CONTAINING 90% GASOLINE *A*

Mixture			Critical water content, % by vol.		
Gasoline *A*	Ethanol	Isopropanol	0° C.	−20° C.	−40° C.
90	10	0	0.25	0.16	0.07
90	8	2	0.31	0.22	0.15
90	7	3	0.35	0.26	0.18
90	6	4	0.38	0.29	0.21

Figs. 5 and 6 give the results of similar experiments carried out with mixtures containing 80 and 70% of gasoline *A* respectively.

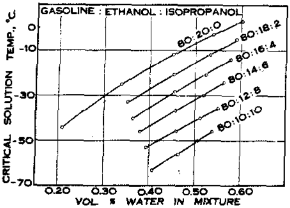

FIG. 5. *Critical water contents of mixtures containing 80% gasoline A.*

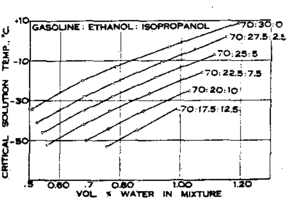

FIG. 6. *Critical water contents of mixtures containing 70% gasoline A.*

For a given temperature the critical water content increases rapidly as the percentage of gasoline in the mixture is decreased. Table V gives the critical water content of these mixtures for temperatures of 0°, −20° and −40° C.

TABLE V

CRITICAL WATER CONTENTS OF MIXTURES CONTAINING 80 AND 70% GASOLINE *A*

Mixture			Critical water content, % by vol.		
Gasoline, %	Ethanol, %	Isopropanol, %	0° C.	−20° C.	−40° C.
80	20	0	0.57	0.38	0.24
80	16	4	0.72	0.53	0.37
80	12	8	0.92	0.71	0.51
70	30	0	1.02	0.68	0.45
70	25	5	1.26	0.89	0.62
70	20	10	1.50	1.11	0.82

Similar experiments were carried out using straight run gasoline *B* which possessed a higher volatility than gasoline *A*. It was found that for a given water content, the critical solution temperatures were lower for mixtures made up from the straight run gasoline having the higher volatility. The results are given in Table VI.

TABLE VI

CRITICAL SOLUTION TEMPERATURES OF MIXTURES CONTAINING 90, 80 AND 70% OF GASOLINE *B* AND VARIOUS PERCENTAGES OF ETHANOL

Mixture			Water content of mixture, % by vol.	Critical solution temp., ° C.
Gasoline, %	Ethanol, %	Isopropanol, %		
90	10	0	0.23	−12
90	9	1	0 23	−18
90	8	2	0.23	−26
90	7	3	0.23	−33
80	20	0	0.45	−21
80	18	2	0.45	−29
80	16	4	0.46	−36
80	14	6	0.46	−44
70	30	0	0.68	−29
70	27.5	2.5	0.67	−35
70	25	5	0.66	−42
70	22.5	7.5	0.65	−49

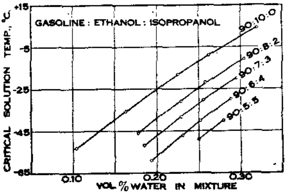

FIG. 7. *Critical water contents of mixtures containing 90% gasoline C.*

Figs. 7, 8 and 9 give the results obtained with mixtures of cracked gasoline *C* with ethanol and isopropanol. As before, the mixtures investigated contained 90, 80 and 70% of gasoline respectively.

It will be seen that the critical water contents of these mixtures are higher

than those of mixtures containing either of the straight run gasolines. This is shown in Table VII by comparison with the results given in Tables IV and V.

TABLE VII

CRITICAL WATER CONTENTS OF MIXTURES CONTAINING 90, 80 AND 70% GASOLINE *C*

Mixture			Critical water content, % by vol.		
Gasoline *C*, %	Ethanol, %	Isopropanol, %	0° C.	−20° C.	−40° C.
90	10	0	0.30	0.22	0.15
90	8	2	0.35	0.27	0.19
90	7	3	0.38	0.30	0.22
90	6	4	0.41	0.33	0.25
80	20	0	0.70	0.50	0.35
80	16	4	—	0.64	0.46
80	12	8	—	0.80	0.60
70	30	0	1.25	0.84	0.59
70	25	5	—	1.09	0.76
70	20	10	—	1.25	1.00

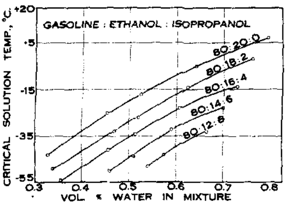

FIG. 8. *Critical water contents of mixtures containing 80% gasoline C.*

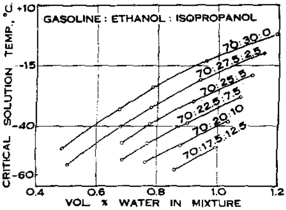

FIG. 9. *Critical water contents of mixtures containing 70% gasoline C.*

A comparison of the critical solution temperatures obtained with straight run gasoline *A* and cracked gasoline *C* is given in Table VIII.

TABLE VIII

COMPARISON OF CRITCAL SOLUTION TEMPERATURES OF STRAIGHT RUN AND CRACKED GASOLINE WITH ETHANOL NO. 8

Mixture			Critical solution temp., ° C.	
Gasoline, %	Ethanol, %	Isopropanol, %	Straight run (*A*)	Cracked (*C*)
90	10	0	− 6	−18
90	8	2	−19	−31
90	5	5	−43	Below −55
80	20	0	−12	−25
80	16	4	−28	−41
80	12	8	−46	Below −55
70	30	0	−20	−33
70	25	5	−35	−47
70	20	10	−50	Below −55

C. Gasoline, Ethanol and Benzene

Gasoline *C* was used in most of the mixtures with ethanol and benzene. The critical solution temperatures for mixtures containing 80% of gasoline C are shown in Fig. 10. Fig. 11 gives the critical water content of four such mixtures at temperatures corresponding to winter conditions. It is evident that the higher the ratio of alcohol to benzene the greater is the critical water content.

Figs. 12, 13 and 14 show the same results for mixtures containing 90, 70 and 60% of gasoline.

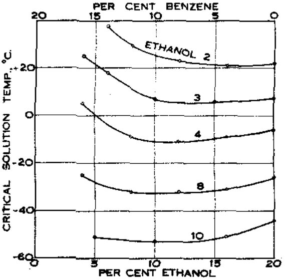

FIG. 10. *Critical solution temperatures of mixtures containing 80% gasoline C.*

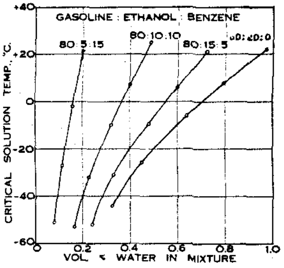

FIG. 11. *Critical water contents of mixtures containing 80% gasoline C.*

Mixtures of gasoline and benzene containing 30 or 40% of the latter deposit crystals at temperatures only slightly below 0° C. The addition of ethanol lowers the crystallizing temperature and if sufficient alcohol is present (depending on the water content), the mixture may develop a cloud before crystallization occurs. The crystallization point is lowered when commercial benzol is used in place of pure benzene as shown in Table XIII.

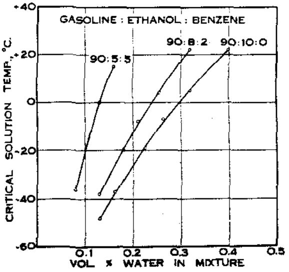

FIG. 12. *Critical water contents of mixtures containing 90% gasoline C.*

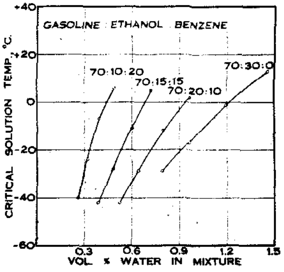

FIG. 13. *Critical water contents of mixtures containing 70% gasoline C.*

With ternary mixtures containing not less than 70% of cracked gasoline, crystallization is not a factor in the temperature range examined. Mixtures having 60% of gasoline and more than 15% of benzene (with ethanol) would be expected to show crystallization when exposed to severe winter temperatures (Fig. 14).

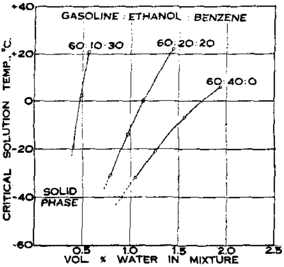

FIG. 14. *Critical water contents of mixtures containing 60% gasoline C.*

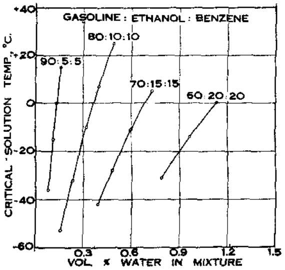

FIG. 15. *Critical water contents of mixtures with gasoline C in which the ratio of alcohol to benzene is 1 : 1.*

When the ratio of alcohol to benzene is kept constant at 1 : 1, the critical water content increases rapidly with decreasing percentages of gasoline (Fig. 15).

The effect of varying two components at a time while keeping the third constant may be considered as follows:—

(a) If the proportion of alcohol to total mixture is constant and the percentage of benzene is increased, the critical water content is increased only moderately as shown in Tables IX and X.

TABLE IX

CRITICAL WATER CONTENT OF MIXTURES CONTAINING 10% OF ALCOHOL

Mixture			Critical water content, % by vol.		
Gasoline *C*, %	Alcohol, %	Benzene, %	0° C.	−20° C.	−40° C.
90	10	0	0.30	0.22	0.15
80	10	10	0.36	0.28	0.20
70	10	20	0.43	0.34	0.26
60	10	30	0.48	0.40	Cryst.

TABLE X

CRITICAL WATER CONTENT OF MIXTURES CONTAINING 20% OF ALCOHOL

Mixture			Critical water content, % by vol.		
Gasoline *C*, %	Alcohol, %	Benzene, %	0° C.	−20° C.	−40° C.
80	20	0	0.70	0.50	0.35
70	20	10	0.94	0.72	0.54
60	20	20	1.12	0.90	—

(b) On the other hand, with mixtures containing a fixed percentage of benzene, the critical water content increases rapidly with increasing percentage of alcohol as shown by Table XI and Fig. 16.

TABLE XI

CRITICAL WATER CONTENTS OF MIXTURES CONTAINING 10% OF BENZENE

Mixture			Critical water content, % by vol.		
Gasoline *C*, %	Alcohol, %	Benzene, %	0° C.	−20° C.	−40° C.
90	—	10	—	—	—
80	10	10	0.36	0.28	0.20
70	20	10	0.94	0.72	0.54
60	30	10	1.52	1.24	—

(c) When the proportion of gasoline to total mixture is constant, the maximum water tolerance would be obtained by using "absolute"* alcohol for the remainder of the mixture and leaving out the benzene entirely (Figs. 11, 12, 13 and 14). The situation is different when the alcohol available for mixing contains water, since the replacement of part of the moist alcohol by dry benzene reduces the water content of the final mixture. Although the critical water content is lowered at the same time, the first effect may more than compensate for the second. For example, a mixture containing 60% of gasoline *C* and 40% of alcohol No. 2 has a critical solution temperature of +6° C. If 20% of the alcohol is replaced by benzene, the critical solution temperature becomes −14° C., a lowering of 20°. The respective water contents and critical solution temperatures are shown in Table XII.

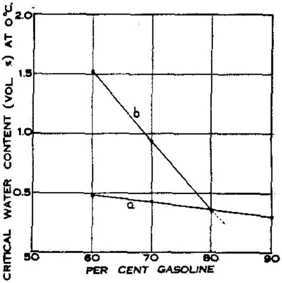

FIG. 16. *Critical water contents at 0° C. of ternary mixtures containing (a) 10% alcohol, and (b) 10% benzene.*

TABLE XII

USE OF BENZENE TO INCREASE WATER TOLERANCE

Mixture			Water content, % by vol.	Critical sol. temp., ° C.	Critical water content at 0° C., % by vol.	Water tolerance at 0° C., % by vol.
Gasoline *C*, %	Alcohol, No. 2, %	Benzene, %				
60	40	0	1.92	+ 6	1.76	—
60	20	20	0.96	−14	1.12	0.16

**Alcohol containing no water.*

When the percentage of gasoline to be used in the mixture and the water content of the alcohol are fixed, there is a definite percentage of benzene which will give maximum water tolerance. This can be found by plotting the actual water contents of possible mixtures against percentage of benzene, as has been done in Fig. 17. The water tolerance of a mixture at a given temperature is defined as the difference between its actual water content and the critical water content at that temperature.

It is readily seen that a mixture containing approximately 10% of benzene will have a greater water tolerance at 0° C. than any other mixture made with 95% (by weight) alcohol* and containing 70% of gasoline *C*. However,

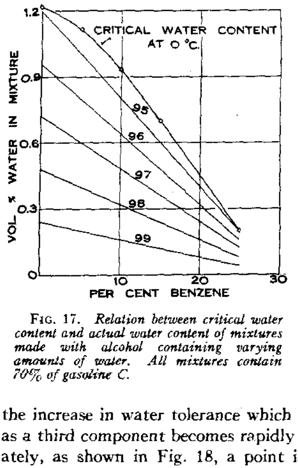

FIG. 17. *Relation between critical water content and actual water content of mixtures made with alcohol containing varying amounts of water. All mixtures contain 70% of gasoline C.*

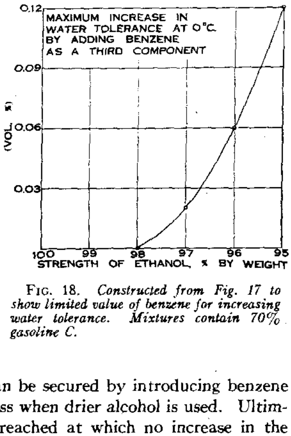

FIG. 18. *Constructed from Fig. 17 to show limited value of benzene for increasing water tolerance. Mixtures contain 70% gasoline C.*

the increase in water tolerance which can be secured by introducing benzene as a third component becomes rapidly less when drier alcohol is used. Ultimately, as shown in Fig. 18, a point is reached at which no increase in the water tolerance is possible, and for the particular conditions stated, the limit is reached when alcohol of approximately 98% strength (by weight) is used. With mixtures containing 60 and 80% of gasoline respectively, a similar limit is observed with alcohol of a strength of between 97 and 98%.

If a mixture containing 90% of gasoline is desired, it appears that the remainder of the mixture may consist entirely of moist alcohol, since the introduction of benzene into the formula gives a negligible increase in water tolerance. Even in those mixtures based on 60, 70 and 80% gasoline, the increase in water tolerance to be obtained by using benzene is small in comparison with that attainable by using alcohol containing 1% less of water.

**See footnote p. 506.*

With gasoline *C* and alcohol No. 4, the critical solution temperatures of mixtures containing commercial benzol are about 3° C. higher than when pure benzene is used, as shown in Table XIII.

TABLE XIII

COMPARISON OF PURE BENZOL AND COMMERCIAL BENZOL

Mixture			Critical solution temp., ° C.	
Gasoline *C*, %	Alcohol No. 4, %	Benzene or benzol, %	Pure benzene	Commercial benzol
90	5	5	+15	+17
80	10	10	−10	− 7
70	15	15	−28	−25
60	20	20	Cryst.	−45
75	10	15	−18	−15

The difference between the critical solution temperatures of mixtures of straight run gasoline *B* and of cracked gasoline *C* is given in Table XIV for alcohols of two strengths.

TABLE XIV

COMPARISON OF STRAIGHT RUN AND CRACKED GASOLINE

Mixture				Critical solution temp., ° C.	
Strength of alcohol (% by wt.)	Gasoline	Alcohol	Benzene	Straight run (*B*)	Cracked (*C*)
96.00	80	20	0	− 1.5	− 6
96.00	80	16	4	− 5	− 9
96.00	80	12	8	− 7	−11
96.00	80	8	12	− 4	− 9
95.08	80	20	0	+12	+ 8
95.08	80	15	5	+10	+ 6
95.08	80	10	10	+11	+ 7
95.08	80	6	14	+22	+18
96.00	90	5	5	+18	+15
96.00	80	10	10	− 5.5	−10
96.00	70	15	15	−25	−28
96.00	60	20	20	Cryst.	Cryst.

(D) Comparison of Isopropanol and Benzene as the Third Component in Gasoline–Ethanol Mixtures

The effectiveness of isopropanol in lowering the critical solution temperature of any given mixture is far greater than that of benzene (Table XV).

TABLE XV

CRITICAL SOLUTION TEMPERATURES OF 90 : 5 : 5 MIXTURES HAVING BENZENE OR ISOPROPANOL AS THE THIRD COMPONENT, GASOLINE *C*

No.	Ethanol strength, % by wt.	Critical solution temp., ° C.	
		Benzene	*Isopropanol*
4	96.00	+15	Below −55
5	96.71	0	Below −55
6	97.18	−15	Below −55

This is further illustrated in Table XVI which includes mixtures containing 80 and 90% of gasoline respectively. In the latter case, the use of benzene as the third component actually raises the critical solution temperature, whereas isopropanol lowers it by more than 50° C.

The critical water contents of various mixtures of gasoline *C* and ethanol together with benzene or with isopropanol are compared in Table XVII.

TABLE XVI

CRITICAL SOLUTION TEMPERATURES OF VARIOUS MIXTURES

Mixture				Critical solution temp., ° C.
Gasoline *C*	Ethanol 4	Third component		
80	20	None		− 6
80	10	Comml. benzol	10	− 7
80	10	C.p. benzene	10	−10
80	10	Isopropanol *A*	10	Below −55
80	10	Isopropanol *B*	10	Below −55
90	10	None		+ 7.5
90	5	Comml. benzol	5	+17
90	5	C.p. benzene	5	+15
90	5	Isopropanol *A*	5	−40
90	5	Isopropanol *B*	5	Below −55

The advantage of isopropanol in increasing the critical water content is clearly shown.

TABLE XVII

CRITICAL WATER CONTENTS OF VARIOUS MIXTURES

Mixture				Critical water content (% by volume)		
Gasoline *C*	Ethanol	Third component		0° C.	−20° C.	−40° C.
90	10	None		0.30*	0.22	0.15
90	5	Benzene	5	0.14	0.10	0.07
90	5	Isopropanol	5	>0.45	>0.35	0.28
80	20	None		0.70	0.50	0.35
80	10	Benzene	10	0.36	0.28	0.20
80	10	Isopropanol	10		>0.83	>0.65
70	30	None		1.25	0.84	0.59
70	20	Benzene	10	0.94	0.72	0.53
70	20	Isopropanol	10	>1.6	>1.3	1.00

Discussion

Absolute ethanol and gasoline mix in all proportions, and mixtures containing 10 to 40% of absolute alcohol are stable at temperatures down to −60° C. or lower. Nevertheless, the addition of a small percentage of water to these mixtures will cause separation into two phases. The critical water content is small at low temperatures and for mixtures containing from 10 to 40% alcohol it varies from 0.1 to 1.0% by volume at −40° C.

The water tolerance of a given ethanol-gasoline mixture can be raised by the addition of a third liquid or "blending agent," and it has been thought that this practice would be desirable in countries where sub-zero temperatures prevail during the winter.

An ideal blending agent should be cheap, effective and available in large quantities. Acetone, ethyl ether and *n*-butanol are considered to be too costly for this purpose. The remaining possibilities appear to be benzene from coal carbonization, and isopropanol, which is being produced at present from waste gases at petroleum refineries.

Benzene may be used to increase moderately the critical water content of ethanol-gasoline mixtures by substituting benzene for part of the gasoline. If the alcohol is of a strength less than 97 or 98% by weight, a slight increase in water tolerance may be obtained by replacing part of the alcohol in the formula by benzene. This is true only if the mixture contains less than 90% of gasoline. The existence of optimal ratios of benzene to ethanol for maximum water tolerance has been demonstrated. While benzene has other properties which make it desirable as a component of mixed motor fuels, its effect in increasing water tolerance appears to be too slight to be of practical value.

On the other hand, isopropanol increases the water tolerance and the critical water content of any ethanol-gasoline mixture to which it is added. If half of the ethanol in a given formula is replaced by isopropanol, the critical water content at −20° C. is increased by about 60%, while at −40° C. it is nearly doubled. Although there is no information available as to the degree of water tolerance desirable in mixed motor fuels, the results given above indicate that mixtures of reasonably high water tolerance may be prepared by the addition of isopropanol to ethanol-gasoline blends.

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HYDROTHERMAL SYNTHESIS OF CALCIUM HYDROSILICATES[1]

By V. A. Vigfusson,[2] G. N. Bates[3] and T. Thorvaldson[4]

Abstract

A crystalline substance which appears in steam-cured Portland cement mortar has been shown to be a calcium hydrosilicate and has been prepared by hydrothermal synthesis from mixtures of silica sand with lime, dicalcium silicate and tricalcium silicate, silica gel and lime (after preliminary steam treatment and ignition) and by the action of saturated lime water on quartz crystals or fused silica plates. The crystals appear not to be acted on by solutions of sodium sulphate, calcium sulphate or alkali hydroxides, but they are slowly decomposed by solutions of magnesium sulphate and alkali carbonates and rapidly by dilute acids and ammonium salts. The crystals were obtained free from amorphous matter by growing them on quartz or silica plates in saturated lime water. When the compound was prepared in this way, the lime-silica-water ratio was found to be 2 : 1 : 1, the formula being therefore $2CaO.SiO_2.H_2O$ or $H_2Ca_2SiO_5$. This product usually appears as thin lath-like prisms showing parallel extinction, positive elongation and moderate birefringence. The crystals are optically positive with a fairly large optic angle. $2V = 68°$. The indices of refraction are $\alpha_{Na} = 1.614 \pm .002$, $\beta_{Na} = 1.620 \pm .002$, $\gamma_{Na} = 1.633 \pm .002$. The optical plane is parallel to the macropinacoid (100) and the acute bisectrix Z is parallel to the direction of elongation which is taken as the crystallographic axis C. The optical properties and X-ray pattern are distinctive and entirely different from those of hillebrandite or foshagite, which have the same composition.

Another crystalline calcium hydrosilicate was obtained by hydrothermal synthesis from excess lime and silica gel. This appeared as very small needle-like prisms, observable only when magnified about 200 times. The crystals show parallel extinction, positive elongation and very low birefringence with an index of refraction of $1.597 \pm .003$. Analysis of this product, extracted with a glycerol-water mixture to remove excess lime, gave a lime-silica ratio of 2 to 1 with an uncertain amount of water of at least one mole. The X-ray pattern is distinctive and shows only slight similarity to the hillebrandite pattern.

Introduction

A preliminary paper (11) described a crystalline substance formed in Portland cement mortar subjected to the action of saturated steam under pressure. Mortars thus treated show an increased resistance to the disintegrating action of sulphate solutions. This effect varies with the temperature and duration of the steam treatment (12, 13) and for a given cement appears to be related to the amount of the observed crystalline product formed. When a Portland cement mortar, made with quartz sand and a cement high in tricalcium silicate, is subjected to the action of saturated steam at say 150° C. and the product examined by the microscope, hexagonal plates of calcium hydroxide are soon observed in the mortar, the amount of these increasing with continued treatment. There is observed in addition the presence of birefringent crystals in the form of thin lath-like plates which increase in number and size as the treatment progresses, while the amount of the hexa-

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gonal plates of calcium hydroxide reaches a maximum and then decreases until they disappear completely. The optical properties of the lath-like plates could not be identified with those of any previously described product.

Preliminary Experiments

Attempts to separate these crystals completely by mechanical means from the cement mortar in which they were embedded were not successful. It was, however, possible to study the action of other substances on the product by means of the microscope, without obtaining a pure sample. The crystals were apparently not attacked by solutions of calcium sulphate or sodium sulphate, but were slowly decomposed by solutions of magnesium sulphate, behaving in these respects similarly to steam-cured Portland cement mortars (13). The crystals were found to be quite stable in solutions of sodium, potassium and ammonium hydroxides, but were readily decomposed by dilute acids and by solutions of ammonium salts. They were slowly decomposed by solutions of normal alkali carbonates with the formation of calcite crystals on the surfaces attacked. Further, with dilute sulphuric acid, gypsum was formed. The new product thus contains considerable calcium.

To observe more exactly the decomposition of the crystals by acids, the following method was used. Some of the hydrated cement containing the crystalline material was separated from the sand grains of the mortar and suspended in a warm dilute solution of gelatin. A drop of this suspension was mounted on a slide with cover glass and cooled till a gel was formed. A drop of the reagent was placed in contact with one edge of the mount while a filter paper was applied to the opposite edge. Thus it was possible to draw the reagent through the preparation slowly enough to make careful microscopic observations. When dilute hydrochloric acid was used, the action progressed slowly from the edges towards the centre of the crystal, leaving an undissolved skeleton form of the original crystal. Experiments with selective dyes (5) and parallel tests with certain silicates and aluminates indicated that the skeleton form of the decomposed crystals consisted mainly of amorphous silica, and that no alumina was present.

Further experiments demonstrated that the crystalline product contained water and lost it only on heating at a high temperature. Thus, heating the hydrated cement containing the crystals at 400° C. for 24 hr. caused a slight apparent roughening of the crystal edges, while prolonged heating at 650° C. rendered the crystals partly isotropic.

These experiments demonstrated that the principal constituents of the new crystalline substance are lime, silica, and combined water, and thus indicated a calcium hydrosilicate. Attempts were therefore made to prepare the crystals from pure materials. A 1 : 10 mixture of pure calcium oxide and quartz sand in a platinum crucible was kept in saturated steam at 150° C. for 48 hr. Microscopic examination of the product showed the presence of a large quantity of the crystals. Crystalline calcium hydroxide was also present, showing that the reaction did not proceed to completion under the conditions of the experiment. However, this method of preparation eliminated all

possibility of the substance containing anything but the oxides of calcium and silicon with combined water. The new calcium hydrosilicate was also obtained by hydrothermal treatment of tricalcium silicate or dicalcium silicate and sand, and also from lime and silica gel (after preliminary hydration and ignition). The product is therefore the result of hydrothermal synthesis under definite conditions, using mixtures of various forms of silica or silicates with lime or with compounds which on hydrolysis yield calcium hydroxide. The synthesis is apparently influenced by temperature, pressure, purity and state of aggregation of the reacting materials and the presence of catalysts such as calcium chloride or sodium chloride. The catalysts appeared to favor the formation of the crystals in certain samples of Portland cement mortar, steam-treated at 150° C.

As this crystalline hydrosilicate is formed on treatment of quartz sand and lime with steam, it may be produced in the process of manufacturing sand-lime bricks. A search of the literature on sand-lime bricks and the hydrothermal formation of silicates gave only meagre information as to the chemical reactions involved or the composition of the products formed. Most workers (2, p. 11; 3, 10) consider that the bonding material is a calcium hydrosilicate of undetermined or variable composition. Some investigators (1, 9) of hydrothermal alteration and synthesis obtained calcium hydrosilicates which they identified as hillebrandite, okenite, and gyrolite. However, the systems usually investigated have been very complex and thus the products have not been homogeneous or readily identified.

While the latter part of the work described in this paper was in progress, reports of extensive researches on the synthesis of hydrosilicates of calcium by Nagai (8) have been published. Mixtures of powdered quartz and calcium oxide in various proportions were treated in saturated steam at temperatures up to 212° C. for 1 to 10 days, and in steam at atmospheric pressure at temperatures up to 1200° C. While mixtures of hydrosilicates were in general formed, preparations were reported composed mainly of: (i) xonotlite ($CaO.SiO_2.\ 0.25H_2O$), (ii) afwillite (of composition $3CaO.2SiO_2.H_2O$ and $3CaO.2SiO_2.8H_2O$) and (iii) hillebrandite ($2CaO.SiO_2.H_2O$).

Preparation of Pure Samples of the Calcium Hydrosilicate

1. *Preparation from Calcium Hydroxide and Quartz Sand*

The quartz sand (20–30 mesh) was treated with dilute potassium hydroxide and then with hot hydrochloric acid. It was finally washed with distilled water till free from chlorides. After all dark or stained grains were discarded, the sand gave, on treatment with hydrofluoric acid, a non-volatile residue of only 0.34%. Calcium oxide, freshly prepared from re-precipitated calcium carbonate by ignition to constant weight at 900° C., was mixed with five times its weight of the prepared sand in platinum crucibles. Water free from carbon dioxide, was added and the samples were stirred to form a thick slurry. The crucibles were placed in an autoclave and exposed to saturated steam at 170° C. for about two weeks. After treatment the samples were dried over

d the sand separated by sieving. The fine powder was extracted times with glycerol-water solution (sp. gr. 1.142) till no free lime e detected by Emley's method (7) or White's test (15). Filtrations rformed in air, free from carbon dioxide. The water used for washing o free from carbon dioxide. The extracted material was carefully to remove all glycerol, dried over lime to constant weight, and anal- The results of the analyses of two preparations are given in Table I.

TABLE I

CAL ANALYSES OF CALCIUM HYDROSILICATE PREPARED FROM SAND AND CALCIUM OXIDE

	1	2	Calcd. for $2CaO.SiO_2.H_2O$
	56.09	57.72	58.95
	None	None	
l Fe_2O_3, %	0.36	Not weighed	
	31.83	30.97	31.58
	10.92	10.64	9.47
	99.20	99.33	100.0
ios			
	1.887	1.997	2
	1.000	1.000	1
	1.144	1.145	1

aration from Lime Water and Quartz Crystals and Silica Plates

tz crystals and fused silica plates were suspended in saturated lime n silver containers, excess lime being placed in the bottom of each er. The samples were treated in an autoclave at 170° C. for periods from 7 to 14 days. The crystals and plates were then removed, washed ater, free from carbon dioxide, and dried over lime. Examination that the surfaces of the quartz crystals and silica plates were studded oups of the orthorhombic lath-like crystals. Some of these crystals 15 × 0.5 mm. or more. Fig. 1 shows a photomicrograph of the sur- one of the silica plates.

taining uniform deposits of well defined crystals, different experiments t equally successful. In some experiments with quartz crystals which eady been treated and the crystalline deposit scraped off, no growth d on the quartz, while a deposit of fine needle-shaped crystals was on the surface of the mass of calcium hydroxide at the bottom of the er. In other cases, when nickel-plated containers were used to hold cting materials, the silica plates were covered with a mixed deposit orthorhombic "laths" and fine needles, while large crystals of the were found attached to the inner surface of the container. In some ients with silica plates in nickel-plated containers a film of very finely ine material of low birefringence, with an occasional orthorhombic imbedded in the mass, was formed on the surface of the silica plates.

This film flaked off easily leaving the silica plates very clean. After drying this material at 105° C. two analyses, which agreed very well, gave a $CaO : SiO_2 : H_2O$ ratio of 0.86 : 1 : 0.56. The X-ray diffraction pattern of this material differed entirely from those of the "laths" and "needles" mentioned above.

Slight variation in conditions may thus have a large effect on the crystal growth. The temperature of the autoclaves varied considerably at times. This would not only affect the concentration of hydrated lime in solution but might also tend to localize differences in the concentration in different parts of the containing vessel. Koyanagi (6) has demonstrated that a change in the concentration of lime affects, within narrow limits, the crystalline form of hydrated tricalcium aluminate deposited from a solution of alumina cement.

The crystals grown on five large quartz crystals and two silica plates were combined and dried in vacuum over freshly ignited lime. Microscopic examination of the sample showed that it was remarkably uniform and free from amorphous matter. Careful microscopic and chemical tests failed to indicate the presence of either carbonate or hydrate of lime. Fig. 2 shows a photomicrograph of some of the crystals and Fig. 3, the same between crossed nicols. Fig. 4 shows some typical crystals with higher magnification.

A microchemical method of analysis was employed, using a Kuhlmann balance (sensitive to 0.001 mgm.), and samples of 6 to 20 mgm. A procedure avoiding any filtration or transfer of material was used. The water was determined by loss on ignition, the calcium by treating the sample with hydrofluoric acid, weighing the calcium as the fluoride, and the silica by difference. The method was tested out on pure samples of calcium carbonate and dicalcium silicate and found to give good results. The results of three analyses by this method given in Table II indicate definitely a 2 : 1 : 1 compound.

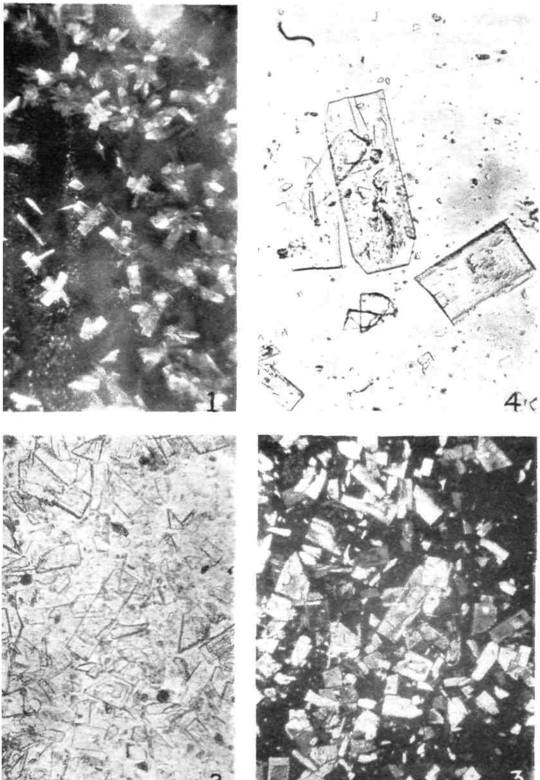
TABLE II

CHEMICAL ANALYSES OF CRYSTALS GROWN ON QUARTZ AND SILICA PLATES

	1	2	3	Calcd. for $2CaO.SiO_2.H_2O$
CaO, %	58.60	58.47	58.45	58.95
SiO_2, %	30.30	30.08	30.36	31.58
H_2O (loss on ignition), %	11.10	11.45	11.19	9.47
Molar ratios				
CaO	2.07	2.08	2.06	2
SiO_2	1.00	1.00	1.00	1
H_2O	1.22	1.27	1.23	1

Physical and Optical Properties of Calcium Hydrosilicate Prepared from Lime Water and Quartz

The optical properties of the orthorhombic crystals found in steam-treated Portland cement mortar have been described by Thorvaldson and Shelton



Surface of fused silica plate, showing crystals of calcium hydrosilicate. Oblique illumin-
× *22.* FIG. 2. *Crystals of calcium hydrosilicate from silica plates and quartz crystals.*
FIG. 3. *Same as Fig. 2 with crossed nicols.* FIG. 4. *Typical "lath" crystals from silica*
× *220.*

(11). The pure crystals obtained from the quartz and silica plates (analysis given in Table II) gave identical results. The optical data and other characteristics are summarized as follows:—

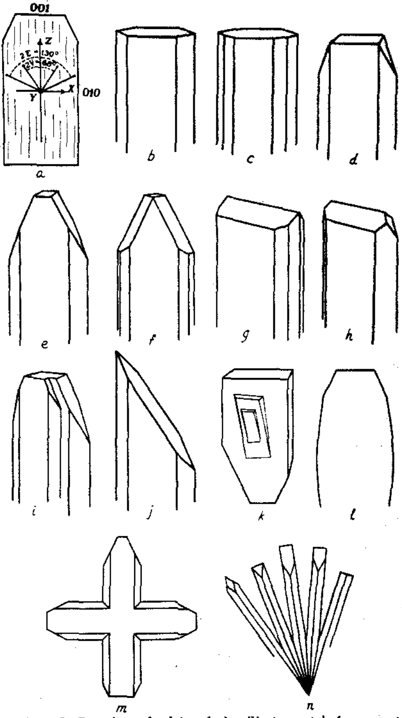


FIG. 5. *Drawings of calcium hydrosilicate crystals from quartz and silica plates. a. Optic orientation and optic angles (2V, 2E) of the illustrated crystals of calcium hydrosilicate. b, c, d, e. Common forms showing basal pinacoids, macropinacoids, prisms, and bracydomes. f. Diagram of form containing pyramids. g and h. Macrodome. i. Parallel growth. j. Hemihedral development. k. Form consisting of pinacoids and bracydomes, with parallel growths on the macropinacoid. l. Barrel-shaped outline of crystals sometimes found in steam-treated cement mortar. m. Cruciform twinning. n. Radial growth.*

The crystals are lath-like or prismatic and grow in groups or radiating clusters from the surface of the quartz. Parallel growths and twinning are fairly common. The crystals are colorless and transparent with shining lustre. They are brittle, have a hardness about 5 and specific gravity, as determined with heavy liquids, close to 2.8. They show prismatic cleavage, parallel extinction and positive elongation. The optical character is positive, $Bx_a = Z$. The direction of elongation, parallel to Z, is selected as the crystallographic axis, C. The optic axial plane is parallel to the macropinacoid (100). Consequently only a flash figure is obtained on the ordinarily flat-lying prisms. Rolling a crystal on end gives an acute bisectrix figure. The optic angle thus observed is large and by calculation from the indices of refraction, $2V = 68°$. Indices of refraction are:—

$\alpha_{Na} = 1.614 \pm .002$,
$\beta_{Na} = 1.620 \pm .002$,
$\gamma_{Na} = 1.633 \pm .002$

Birefringence $\beta - \alpha = 0.006$, $\gamma - \alpha = 0.019$

Pleochroism and dispersion are not evident. Drawings shown in Fig. 5 *(a,* *(b)*, *(c)*, *(d)* and *(e)* represent the optic orientation and the forms commonl observed, while *(f)* illustrates the rare occurrence of the pyramid (111) an *(g)* and *(h)* of the macrodome (101). Radiating or parallel growths an twinning occurring frequently are represented by *(n)*, *(i)*, *(k)* and *(m)*. I steam-treated Portland cement mortar curved growth results in the form ation of barrel-shaped crystals illustrated in *(l)*. Hemihedral growth i indicated by the rare occurrence of a crystal such as *(j)*. Doubly terminate crystals show, however, that the symmetry is definitely holohedral.

Calcium Hydrosilicate Prepared from Lime and Silica Gel

Mixtures of pure lime and silica gel* in molar ratio of 4 : 1 to give a larg excess of lime, were prepared and treated with saturated steam as describe above for the lime and sand preparation, except that there were severa treatments with intermediat grinding of the samples. Th preparations were extracte with a glycerol solution t remove the excess of fre lime, washed and dried at 105 C. to constant weight. Th product was fairly homo geneous, and consisted of fin needle crystals instead of th lath-like plates describe above. The material differe also in optical properties an X-ray diffraction pattern Results of analysis in duplicate of two preparations are given in Table III.

TABLE III

ANALYSIS OF CALCIUM HYDROSILICATE OCCURRING AS NEEDLE-SHAPED CRYSTALS

Sample Total time in autoclave (days)	1 32	2 26
CaO, %	58.27	57.57
SiO_2, %	29.30	28.52
Loss on ignition, %	11.76	12.86
	99.33	98.95
Molar ratios		
CaO	2.13	2.16
SiO_2	1.00	1.00
H_2O	1.34	1.50

The extremely fine subdivision of the material no doubt favored the ad sorption of both calcium hydroxide and of water. Further, the exposure t air during grinding and during the extraction of the excess of free lime gave in spite of precautions, opportunity for the formation of some calcium car bonate through the absorption of carbon dioxide by the free calcium hydroxide These samples were therefore not as pure as those obtained by growing crystal on quartz and silica plates. The analyses, however, indicate a compounc with a lime-silica ratio of 2 : 1 with one mole or possibly more of water Slightly high values for calcium oxide and for loss on ignition are to be expected

Optical Properties of Needle Crystals from Lime and Silica Gel

The crystals occurred usually in radiating clusters or masses and als detached. They are anisotropic, very weakly birefringent, with positive elongation and parallel extinction. With the oil immersion they are found to be thin prisms usually having needle-like or incomplete terminations Some twinning and parallel growths were observed. No interference figures

**The silica gel passed through a 300-mesh sieve and contained 87.1% SiO_2. Loss on ignition was 12.5%, and non-volatile residue with hydrofluoric acid, 0.4%.*

were obtainable so the optical character was not determined. The mean index of refraction was found to be 1.597 ± .003. The refractive index is thus decidedly lower than the indices for hillebrandite, but close to those of foshagite. The other optical properties, as far as they were determined, are similar to those of the natural minerals. The X-ray diffraction pattern (Table IV and Fig. 6), however, indicates that these crystals represent another distinct form of hydrated dicalcium silicate.

TABLE IV

X-RAY DATA

Material obtained from steam treated Portland cement mortar		Calcium hydrosilicate crystals prepared from saturated lime water and quartz crystals		Calcium hydrosilicate crystals prepared from excess lime and silica gel		Hillebrandite. Sample from Velardena Mine, Durango, Mexico, according to Vigfusson (14)		Calcium hydroxide according to Harrington (4)	
Planar spacing d_{hkl} in Å	Estimated relative intensity	Planar spacing d_{hkl} in Å	Estimated relative intensity	Planar spacing d_{hkl} in Å	Estimated relative intensity	Planar spacing d_{hkl} in Å	Estimated relative intensity	Planar spacing d_{hkl} in Å	Estimated relative intensity
3.51	W	3.53	W	3.50	W	3.51	W		
3.27	SS	3.28	SS	3.32	WWd	3.34	M		
						3.13	W	3.11	M
3.08	M	3.08	WW	3.05	W				
				2.93	SS	2.93	SS		
2.87	Md	2.88	M						
2.80	Md	2.82	M	2.77	W	2.79	M		
2.72	Md	2.68	M	2.67	M	2.68	W		
2.60	Md	*2.60*	M					2.63	SS
2.52	Md	2.53	W			2.46	M		
2.42	S	2.42	S	2.38	M	2.38	WW		
2.30	Md	2.32	M			2.30	M		
2.25	Md	2.24	M	2.26	S	2.22	W		
2.17	WW	2.16	W			2.16	M		
2.07	Sd	2.07	S	2.07	W	2.10	W		
2.00	Md					2.04	M		
1.987	Md	*1.957*	M	*1.958*	S	*1.960*	M		
1.927	Md	1.925	M	1.872	S	1.875	W	1.930	S
		1.845	W	1.810	S	1.820	S		
1.785	S	1.786	S	1.763	W			1.795	S
1.717	M	1.724	Wd	1.714	WW	1.737	S		
						1.675	WW	1.685	M
1.647	S	1.650	S	1.633	Wd	1.623	WW		
1.563	Md	1.566	M			1.595	M		
				1.548	Wd	*1.560*	WW	*1.555*	W
1.535	Md	1.534	M			1.528	M		
				1.518	Wd	1.507	W		
1.475	Wd	1.475	W	1.469	WW	1.467	W	1.480	M
1.446	Wd	1.443	W			1.442	W	1.450	M
				1.415	WW	1.432	W		
1.400	Wd	1.400	W	1.363	W				
1.352	Wd	1.355	M	1.333	WW	1.350	W		
1.305	WW	1.308	W			1.301	W	1.312	M
				1.284	WW	1.262	M		
1.250	Wd	1.246	W						
1.230	Wd	1.228	W	1.223	WW	1.210	W		
				1.178	S	1.183	M	1.175	WW
1.156	Md	1.160	M	1.152	WW	1.149	M	1.144	M
1.138	Md	1.138	M						
				1.122	W	1.121	M	1.128	W
1.112	WW	1.110	W						
1.090	WW	*1.088*	WW	*1.090*	Wd				
				1.059	WWd	1.060	M	1.061	M
1.038	WW	1.040	WW	1.032	WW	1.038	M	1.038	W
1.010	WW	1.008	WW					0.013	W

Estimated relative intensity: SS = very strong; S = strong; M = medium; W = weak; WW = very weak; d = diffuse.

X-ray Data

The products were studied by the powder method. Photographs of the X-ray diffraction patterns were made with a General Electric Company multiple spectrograph. A Coolidge tube with molybdenum target was the source of the X-rays and a zirconia filter was used in front of the film. Table IV gives the planar spacings and estimated relative intensity of the diffraction lines. Pure sodium chloride was used as a standard to correct the planar spacings. For comparison, patterns for hillebrandite (14) and for calcium hydroxide (4) are included in the table. The data in Table IV were charted in Fig. 6 for ready comparison, the height of the lines corresponding to the relative intensity of the diffracted X-ray beam. Examination of the data shows that the pattern for the orthorhombic crystals agrees well with the pattern obtained with material from steam-treated mortar, but is entirely different from the patterns given by calcium hydroxide (4), hillebrandite and foshagite (14), or other known calcium hydrosilicates. The pattern for the "needle" crystals shows a slight similarity to the one for hillebrandite but is in general different.

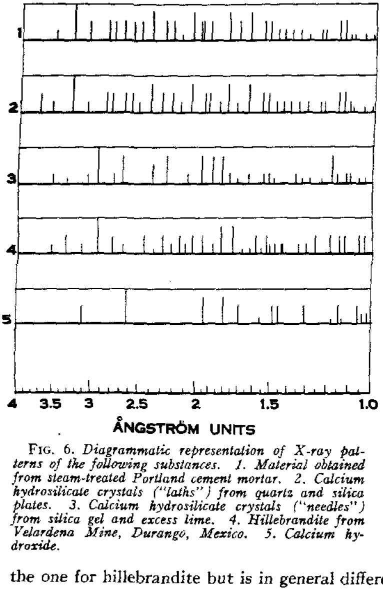


FIG. 6. *Diagrammatic representation of X-ray patterns of the following substances. 1. Material obtained from steam-treated Portland cement mortar. 2. Calcium hydrosilicate crystals ("laths") from quartz and silica plates. 3. Calcium hydrosilicate crystals ("needles") from silica gel and excess lime. 4. Hillebrandite from Velardena Mine, Durango, Mexico. 5. Calcium hydroxide.*

Acknowledgments

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VAPOR DENSITY OF SULPHUR DIOXIDE[1]

By W. W. Stewart[2] and O. Maass[3]

Abstract

The technique developed in this laboratory for measuring gas densities has been made still more sensitive by using a 50-litre volume. The experimental results obtained showed that the apparent molecular weight-pressure isothermal for sulphur dioxide is not a straight line. This is in agreement with the equation of state for gases at low pressures, the study of which has been the main object of this investigation. From the data on sulphur dioxide its true molecular weight was found to be 64.075.

Introduction

The density of sulphur dioxide has been measured at 25° C. and at and below atmospheric pressure and at 0° C. and atmospheric pressure by a method developed in this laboratory. The measurements are correct to within 0.01%. The details of the experimental arrangement have been discussed by Cooper and Maass (2). In the present communication a modification of their apparatus, the introduction of a 50-litre constant volume, will be discussed, as well as corrections which have been applied to the measurements of pressure and mass.

The following units have been used in the calculations and the standardization. With the exception of the value for the density of water these constants are those given by Birge (1): $g_{45} = 980.616$, 1 litre = 1000.027 cm³., ice point = 273.18° A., $d(H_2O)$ at 24.90° C. = 0.9970995 gm./ml., $R = 0.082051$ litre atm./moles °A.

Experimental Work

The largest constant volume used in this method by previous workers was five litres. In the present work the constant volume has been increased to about 50 litres, which has increased the precision of the measurement of the mass of the gas and also reduced the adsorption correction. This volume has been calibrated to within 1 part in 100,000.

The constant volume was constructed from a well aged Pyrex balloon flask. The neck was drawn down to a 1 cm. tube and after completing the calibration a 1 cm. bore stop cock was sealed to this tube.

After carefully cleaning and drying the inside of the constant volume it was mounted in a 45 gallon oak cask which served as a constant temperature bath. The water in the bath was well stirred and the temperature of the bath could be controlled to within 0.002° at 25° C.

It is quite evident that the usual method of calibrating volumes by direct weighings is not feasible for such a large volume. The method adopted for the calibration is independent of the gas laws and depends upon filling the flask up to a definite mark with a known weight of air-free distilled water. The experimental arrangement for filling the constant volume is shown in Fig. 1

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The weight pipette had a capacity of about 5000 gm. A ground glass cap fitted the end of the delivery tube and the stop cock was closed during the weighings. The pipette was filled with air-free distilled water. It was placed in the balance case for half an hour and then weighed on both pans of the balance. The mean of these two weights was taken as the weight of the full pipette. As the water syphoned into the constant volume it was replaced by dry air free from carbon dioxide. The empty pipette was weighed in a manner similar to that described above. This procedure was continued until the constant volume was almost filled. The final addition of water was made from a 100 gm. weight pipette.

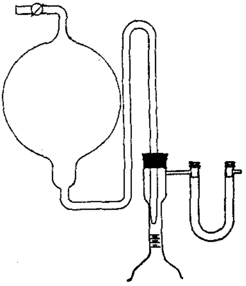

FIG. 1. *Calibration weight pipette.*

During the additions of water to the constant volume the temperature of the constant temperature bath was held at 24.90° C. It was assumed that the temperature of the water in the constant volume was at the temperature of the bath when the position of the meniscus of the water in the neck of the flask remained in a steady state. The position of the meniscus was observed with a reading telescope and was recorded in reference to graduations etched on the neck of the volume. This position was used as the reference mark in further calibrations.

The U-tube shown in the diagram was filled with "Dessichlora". It was weighed before and after the addition of water to the constant volume. From these observations the weight of water lost by evaporation was calculated.

The constant volume was calibrated up to the reference mark by two independent determinations. Then the stop cock was sealed to the neck of the balloon and the volume of the neck above the reference mark up to the top of the stop cock was determined by adding a weighed amount of water from a small pipette.

The large weight pipette was weighed on a 10 kgm. balance. The sensitivity of balance under a 5000 gm. load was 0.010 gm. per 0.5 scale divisions. The weights were compared against a standard set of weights directly up to 50 gm. and then by intercomparison in the usual manner. During each weighing the barometric pressure, temperature and humidity of the balance room were observed, so that each individual weight could be reduced to the vacuum standard.

The results of the two determinations of the volume at 24.90° C. are given in Table I.

The volume of the constant volume at 24.90° C. used in the calculations as V was 48.3184 ± 0.0004 litres.

TABLE I

CALIBRATION DATA FOR THE CONSTANT VOLUME

—	1	2
Weight of water added to flask (*in vacuo*), gm.	48160.67	48161.64
Weight of water lost by evaporation, gm.	0.13	0.36
Net weight of water in flask, gm.	48160.54	48161.27
Volume of flask to the reference mark, ml.	48300.64	48301.37
Mean volume of flask to the reference mark, ml.	48301.00 ± 0.37	
Volume of the neck and the stop cock, ml.	17.37	17.41
Mean volume of the neck and stop cock, ml.	17.39 ± 0.02	
Total mean volume of the flask at 24.90° C., ml.	48318.39 ± 0.39	

It is necessary to apply two corrections to this value to obtain the correct volume corresponding to the temperature and pressure of any individual set of observations. The first is a correction for the temperature coefficient of the Pyrex balloon. This has been calculated from the equation given by Keyes (6). The second is a correction for the contraction of the volume due to a difference in pressure on the constant volume when the gas is at a pressure below atmospheric. This correction was determined by experiment using the method of Rayleigh. The results shown in Table II were obtained for the contraction in volume at various differential pressures. These measurements show that the decrease of the volume of the balloon is a linear function of the differential pressure, which agrees with the results of Moles and Mirvalles (8).

TABLE II

THE CONTRACTION IN VOLUME AT VARIOUS DIFFERENTIAL PRESSURES

P, cm.	75	50	25	10
V, ml.	7.37	4.91	2.44	0.97

The measurement of the pressure of the gas and the corrections applied to reduce the observed pressure to 0° C. 45° Lat. and sea level have been described by Cooper and Maass (3). In the present work an additional correction to the observed pressure has been introduced. The pressure required in the calculation is the mean pressure in the constant volume, that is, the pressure on a horizontal plane passing through the centre of the volume. Therefore, the mercury meniscus on the gas side of the manometer should lie in this plane. In the actual experimental arrangement this meniscus was about 38 cm. above the horizontal plane, so the actual pressure registered on the manometer was less than the pressure at the centre of the volume by an amount which is due to the variation of the pressure with height.

This correction was calculated for each pressure measurement from the equation

$$\log. p_0/p = MH/RT \times 1000 \times 1033.3,$$

where p_0 is the pressure at the centre of the balloon and p the observed pressure; H is the difference in height in cm. between the mensicus and the centre of the balloon. M, the molecular weight of the gas, was calculated

from the ideal gas laws using the uncorrected pressure. At 25° C. and 250 mm. pressure, the correction amounts to 0.054 mm. and at 750 mm. pressure it is 0.145 mm. The error in the correction due to the method of calculation was less than 1% in every observation.

The weight of the gas is obtained by condensing the gas from the constant volume into small weighing tubes which are sealed off and weighed at room temperature. The tubes were shaped similarly to those used by Cooper and Maass (2). They were blown from 2 mm. wall Pyrex tubing. The capillary stem was of about 1 mm. bore and 2 mm. wall. These tubes were strong enough to stand the pressure of the condensed gas at room temperature and no special precautions were taken in handling them. The size of the tubes depended upon the weight of the gas condensed from the constant volume. The volumes of the tubes were about 60, 100, and 175 cc.

The technique of condensing the gas into the tubes was somewhat different from that of previous workers. After carefully cleaning the surface of the tubes, the first stage of the condensation was done by using solid carbon dioxide-ether mixture until the pressure of the gas was reduced to about 2 cm.; then the remaining portion of the gas was condensed into the tube by using liquid air as the refrigerant. If only liquid air was used, the condensation was very slow as the tubes filled with fine needles of solid sulphur dioxide.

After sealing off the tubes they were placed in a cabinet conditioned with dry air until they were in equilibrium with room temperature. The tubes were left in the conditioning cabinet for at least 12 hr. before being weighed directly on the balance pans.

Since in all cases the weight of the gas was of the order of 40 gm., the weighing of these tubes presented no serious difficulties, and could be made with a high degree of precision by observing the usual precautions necessary in handling a chemical balance.

The observed weight of gas was reduced to vacuum standard in each case as outlined by Cooper and Maass (2). An additional correction to the weight of the gas has been introduced, that is, the correction for the amount of gas adsorbed on the surface of the constant volume at the initial pressure. If this factor is neglected the calculated apparent molecular weight is too large, as this adsorbed gas did not contribute to the initial pressure but was condensed out when the pressure was reduced to almost zero pressure. Therefore the amount of gas adsorbed on the surface of the volume has to be subtracted from the observed weight of gas to obtain the true apparent molecular weight. Moles (7) has pointed out that this correction for adsorption has to be considered in all physico-chemical methods for determining molecular weights of gases, and recommends that the size of the volumes be about 1000 cc., as the adsorption correction is reduced by increasing the radius of the balloon. This is about the limit of the volume that can be conveniently handled in the "classical balloon method". By using a constant volume of approximately 50 litres the adsorption correction has been reduced almost to the precision required for the measurement of mass of the gas.

The amount of sulphur dioxide adsorbed on the surface of the constant volume at various pressures has been calculated from the data of Crespi and Moles (5). The data in Table III are those used in the adsorption correction at 25° C.

TABLE III

INFLUENCE OF PRESSURE ON AMOUNT OF SULPHUR DIOXIDE ADSORBED ON THE GLASS SURFACE

P, atm.	1	1/2	1/3	1/4	1/5	1/6	1/7	1/8
SO_2 adsorbed, gm. $\times 10^4$	53.0	37.6	30.6	25.6	23.7	21.8	20.0	18.7

The temperature of the gas is required to within 0.01° C. in order to retain the precision desired in the calculation of M'. The temperature observed at 25.00° C. and at the ice point were well within this limit. At 25° C. the bath was maintained to within 0.002° C. during the run. The temperature of the bath was read on a mercury standard thermometer, PTR 3191, graduated in 0.1° C. In the runs at 0° C. the bath was filled with snow and water. The circulation of the bath liquid was vigorous, and no variation of temperature could be detected by placing a Beckmann thermometer at various positions in the bath. The temperature of the bath remained constant to within 0.002° C. The temperature was determined by a Beckmann thermometer which had been calibrated in a freezing point apparatus, using freshly distilled water, immediately before and after a run.

The sulphur dioxide was obtained from a tank of c.p. material. The gas was purified by fractional distillation under vacuum in an air-free apparatus as described by Cooper and Maass (3).

Experimental Results

The experimental results are shown in Table IV, and graphically in Fig. 2. The last column of the table records the values of the apparent molecular weight for the temperatures and pressures shown in Columns 1 and 3. In the fifth column, w is the weight of the gas corrected for adsorption. The graph shows the mean value of the apparent molecular weight as a function of the pressure. The isothermals are seen to possess a slight curvature, which is contrary to what is expected from the results of other workers. Previously the isothermals for sulphur dioxide were found to be straight lines to within the experimental

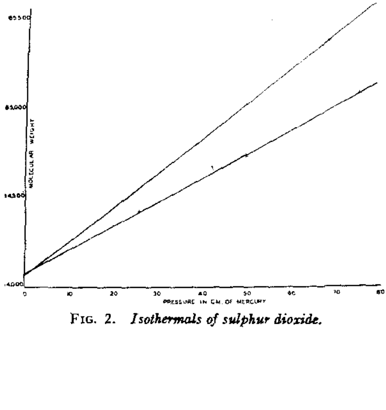

FIG. 2. *Isothermals of sulphur dioxide.*

TABLE IV

ISOTHERMALS AT 25 AND 0° C.

Pressure, mm. Hg.	Residual press., mm.	Temp., °A	V, litres	w, gm.	M', mol. wt.
A. Isothermal at 25° C.					
749.330	0.700	298.18	48.3184	126.646	65.1006
742.449	0.540	298.18	—	125.497	65.0892
753.762	0.787	298.18	—	127.390	65.1049
Mean pressure, 748.514 mm.; mean mol. wt., 65.0982; deviation of mean, 0.0007%.					
482.614	1.207	298.18	48.3156	80.9661	64.7208
493.010	0.270	298.18	48.3157	82.5420	64.7441
498.678	0.820	298.18	48.3151	83.7563	64.7440
505.266	0.300	298.18	48.3158	84.9596	64.7492
Mean pressure, 494.892; mean mol. wt., 64.7395; deviation of mean, 0.0028%.					
253.026	0.255	298.18	48.3135	42.3112	64.4218
258.988	0.508	298.18	—	43.2677	64.4216
258.411	0.519	298.18	—	43.1762	64.4187
256.653	0.700	298.18	—	42.9533	64.4267
258.734	1.146	298.18	—	43.1210	64.4261
256.364	1.570	298.18	—	43.6601	64.4334
252.701	3.354	298.18	—	41.7382	64.4194
Mean pressure, 256.411 mm.; mean mol. wt., 64.4239; deviation of mean, 0.0025%.					
B. Isothermal at 0° C.					
705.694	0.415	273.15	48.3059	130.894	65.4411
649.590	0.425	273.15	48.3055	120.316	65.3532

The calculation of M' has been based on the equation $M' = wRT/PV$. A typical calculation is given in Table V. This shows the results of a complete experiment together with all the corrections necessary in the calculations.

TABLE V

EXAMPLE OF EXPERIMENTAL DATA AND CALCULATION OF M'

Temperature of constant volume, ° C.		25.0	
Temperature of cathetometer scale, ° C.		24.9	
Temperature of manometer bath, ° C.		28.48	
Initial pressure, mm. of Hg.	254.15	245.16	254.155
Residual pressure	0.26	0.25	0.255
Volume of dead space, litres		0.150	
Volume of constant volume at 25.00° C. and $P = 50$ cm., litres		48.3135	
Weight of tube and gas, gm.		106.7848	
Weight of tube and air, gm.		64.5510	
Weight of gas uncorrected, gm.		42.2338	
Weight of tube and distilled water, gm.		137.297	
Volume of tube, cc.		7.95	
Weight of air in tube, gm.		0.0863	
Weight of air displaced by weights, gm.		0.0059	
True weight of gas *in vacuo*, gm.		42.3142	
Wt. of gas adsorbed at 25 cm. on the surface of volume flask, gm.		0.0030	
Temperature correction to pressure, mm.		−1.192	
Level correction to pressure, mm.		0.054	
Gravity correction to pressure, mm.		0.009	
Pressure corrected to 0° C. 45° Lat. and sea level, mm.		253.026	
Corrected residual pressure, mm.		0.254	
Weight of gas corrected for adsorption, gm.		42.3112	
Apparent molecular weight, M'		64.4218	

M' has been calculated in all cases from Equation (1), which is based on the following.

The weight of gas in the constant volume V under a pressure p and a temperature T is $W = PVM'/RT$, where M' is the apparent molecular weight of the gas.

Similarly, the weight of the gas in the constant volume V at a temperature T and residual pressure p is $w = pV'M''/RT$, and the weight of gas in the dead space v at the residual pressure p and temperature t is $w'' = pvM''/Rt$.

If the weight of the gas adsorbed at a pressure P is w''' and the weight of the gas condensed from the volume, corrected to *vacuo*, is W', then $W = W' + w + w'' - W'''$. Therefore

$$M' = \frac{RT(W'' - w''')}{PV} + \frac{pV'M''}{PV} + \frac{pvM''T}{PVt} \quad (1)$$

The value of M'', the apparent molecular weight of the gas at the small residual pressure p, is very nearly that of the true molecular weight of the gas. In all calculations M'' is taken as 64.06.

V' is the volume of the constant volume corrected for a differential pressure of 75 cm. The value of V' used is 48.311 litres.

For the experimental data given above

$$M' = 64.3571 + 0.0645 + 0.0002 = 64.4218 \ .$$

Discussion

The maximum error in the apparent molecular weight estimated from deviations assigned to the four measurements, pressure, temperature, weight and volume, varied at 25° C. and 0° from 0.014% at the highest pressure to 0.027% at the lowest pressure. An examination of the data in Table IV (A) will show that these are maximum deviations between any single value of the molecular weight and the mean value, if the values be compared at the same temperatures and pressures.

The average deviation of the mean of the molecular weight is shown in the table. The value of the molecular weight at any point near the mean was corrected to its value at the mean pressure, then the average deviation calculated in the usual manner. These data indicate that the mean values of the molecular weight are correct to less than 0.01%.

The three values obtained for the 25° C. pressure isothermal do not lie on a straight line. A straight line drawn through any two points will miss the third by an amount many times the experimental error. This result is not unexpected. The equation which has been developed in connection with this work (4) namely,

$$\frac{M'}{M_o} = 1 + \left(\frac{a - \frac{RTb_o}{1+KT}}{R^2T^2}\right)P + 2\left(\frac{a - \frac{RTb_o}{1+KT}}{R^2T^2}\right)^2 P^2 + \ldots\ldots \quad (2)$$

can conveniently be written as

$$\frac{M'}{M_o} = 1 + SP + 2S^2P^2 + \ldots\ldots$$

For carbon dioxide the valves of a, b_o and K are such that S^2 becomes insignificant for pressures of an atmosphere and less. With sulphur dioxide this is not the case. Taking Cooper and Maass' (3) data on sulphur dioxide, it can be shown that the value for S is such that the third term is significant.

The general equation of the isothermal is

$$M' = M_o + bP + CP^2 \tag{3}$$

or

$$\frac{M'}{M_o} = 1 + \frac{bP}{M_o} + \frac{CP^2}{M_o} \tag{4}$$

From Equations (2) and (4), $C = \frac{2b^2}{M_o}$, so the equation for the isothermal is

$$M' = M_o + bP + \frac{2b^2}{M_o}P^2 \tag{5}$$

The values for M_o and b which fit the experimental data most accurately were determined in the following way. From the graph a tentative value for M_o was obtained and the value of the mean molecular weight at the highest pressure was assumed to be correct. From these two values, b was calculated from Equation (5). Then M' was calculated, using the above constants in Equation (5), for the other observed pressures. From an examination of the deviation between the observed molecular weights and the calculated ones, the constants M_o and b could be varied to give an equation that represented the experimental data more accurately. Table VI shows the results calculated from three sets of constants and the deviation between the calculated and observed data.

TABLE VI

CALCULATION OF THE EQUATION OF THE ISOTHERMALS

P, mm.	M' obs.	M' calc.	Deviation	
$M' = 64.0800 + 1.0029\,P + 0.03130\,P^2$				
256.411	64.4239	64.4219	+0.0020	0.0031%
494.892	64.7395	64.7461	−0.0066	0.0010%
748.514	(65.0982)	65.0982	+0.0000	
$M' = 64.0750 + 1.0075\,P + 0.03168\,P^2$				
256.411	64.4239	64.4185	0.0054	0.0084%
494.892	64.7395	64.7446	−0.0051	0.0079%
748.514	(65.0982)	(65.0982)		
$M' = 64.0650 + 1.0170\,P + 0.03229\,P^2$				
256.411	64.4239	64.4152	0.0087	0.013%
494.892	64.7395	64.7429	−0.0034	0.005%
748.514	(65.0982)	(65.0982)		

The equation $M' = 64.0750 + 1.0075\,P + 0.03168\,P^2$ represents the observed data to within the maximum error of each mean molecular weight. It represents the data better than the other two equations do. The value M' 65.0982 at $P = 74.8$ cm. was assumed to be correct for purposes of calculation. This value may have a maximum error of ± 0.0008 as shown in Table IV (A). If M' (65.0982) is varied by -0.0008 the variation in the calculated M_o(64.0750) is ± 0.0006; and b(1.0075) varies by ± 0.0005. Therefore, the isothermal of the molecular weights of sulphur dioxide at 25° C. can be represented in the equation

$$M' = 64.075 + 1.0075\,P + 0.03168\,P^2.$$

The equation for the zero degree isothermal is found to be

$$M' = 64.0750 + 1.4262\,P + 0.06344\,P^2\ ,$$

by putting $M_o = 64.0750$, as obtained from the 25° C. isothermal and calculating the numerical constants from the zero degree data in the same way as in the case of the 25° C. isothermal.

A comparison with the data of Cooper and Maass (3) is made by using the equations obtained for the isotherms and calculating the value of M' for pressures corresponding to their data. In making their calculations they omitted to consider the adsorption correction already described. Furthermore, they used for R the value 0.082046, which is the value for the normal atmosphere, while their pressures were expressed in terms of the atmosphere at 45° Lat. Consequently, their results have been recalculated making a correction for adsorption and using the correct value of R corresponding to the atmosphere at 45° Lat. These recalculated values are used for the purpose of a comparison with the results obtained in the present work. The figures are shown in Table VII.

TABLE VII

COMPARISON OF DENSITY DATA

T, ° A.	P, cm. Hg.	Cooper and Maass	Present work	Difference
273.18	71.496	65.475	65.473	+.002
	43.560	64.905	64.896	+.009
	24.074	64.552	64.533	−.019
298.18	68.856	65.023	64.013	+.010
	45.956	64.697	64.698	−.001
	23.659	64.410	64.391	+.009

It is seen that the agreement in all cases is within the limits of experimental error. This indicates that Cooper and Maass' assumption that the isotherm is a straight line was erroneous. Actually the data conform much better to the equation for a curved isotherm obtained in the present work.

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THE EVOLUTION OF CARBON DIOXIDE IN THE A.-C. ELECTROLYSIS OF SODIUM CARBONATE AND BICARBONATE SOLUTIONS, AND THE DISCHARGE POTENTIALS OF CARBONATE AND BICARBONATE IONS[1]

By J. W. Shipley[2]

Abstract

The a.-c. electrolysis of sodium carbonate solutions at voltages as high as 110, even when arcing occurs on the electrodes, does not cause the evolution of carbon dioxide. In the a.-c. electrolysis of aqueous bicarbonate solutions with platinum electrodes, hydrogen, oxygen and carbon dioxide are evolved freely until all the bicarbonate has been transformed to carbonate, after which the evolution of carbon dioxide ceases and only hydrogen and oxygen are given off. In a.-c. electrolysis of sodium bicarbonate solutions and solutions of the sodium salts of aliphatic acids, a deposit of finely divided platinum is formed on the electrodes. This deposit inhibits the evolution of carbon dioxide, hydrogen and oxygen, but does not affect the current flow. The decomposition potential of bicarbonate solutions in respect to the evolution of carbon dioxide on smooth platinum and with d.c. was found to be 2.2 volts, and of carbonate solutions, 3.5 volts. The anodic discharge potential of HCO_3^- is -1.45 to -1.50 volts, and of CO_3^{--}, -1.90 to -1.95 volts. The evolution of carbon dioxide does not appear to cause any polarizing effect on the anode.

Experimental

During the a.-c. electrolysis of aqueous solutions of the sodium salts of aliphatic acids, using platinum electrodes, carbon dioxide was found to be one of the components of the gaseous products of electrolysis. An examination of the residual solutions disclosed the presence of carbonates and bicarbonates in solution. These had been formed from the sodium organic salt during electrolysis. Before proceeding further it was decided to observe the behavior of pure sodium carbonate and bicarbonate solutions electrolyzed at 110 volts and 60 cycles a.c.

A.-C. Electrolysis of Sodium Carbonate and Sodium Bicarbonate Solutions

Carbon dioxide was not evolved when solutions of various concentrations of freshly fused sodium carbonate in water free from carbon dioxide were electrolyzed at 110 volts a.c. and currents as high as 7.2 amp. per sq. cm. on platinum electrodes. Hydrogen and oxygen were evolved freely as in the a.-c. electrolysis of water (5) containing sodium hydroxide. Even when the temperature was allowed to approach the boiling point of the solution and with arcing occurring on the electrodes no evolution of carbon dioxide could be detected.

Carbon dioxide was evolved copiously from a sodium bicarbonate solution, electrolyzed under similar conditions, at all dilutions and at temperatures as low as 18° C., at 2.5 volts. At low current densities (0.5 amp. per sq. cm.) the evolution of carbon dioxide was small.

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As electrolysis proceeded the electrodes became covered with a finely divided, loosely adhering coating assumed to be platinum black. As this deposit grew, the evolution of gases diminished and finally ceased, without affecting the flow of current. Even at high current densities this phenomenon was observed unless arcing on the electrodes resulted in the loosening of the deposit and its removal from the electrode surface. When this occurred, evolution of gas recommenced and continued until a new deposit was built up. Apparently the products of electrolysis recombined on the surface of the electrodes.

When the evolved gases (hydrogen, oxygen and carbon dioxide), collecting in the electrode chamber above the electrolyte, were left standing for a few hours, they recombined completely, sometimes with a slight explosion. The reaction is attributed to the catalytic effect of the finely divided platinum adhering to the upper walls of the electrode chamber, with which the gases were in contact.

Continued a.-c. electrolysis of sodium bicarbonate solution resulted in a mixture of carbonate and bicarbonate, the proportions depending on the rate of evolution of carbon dioxide, and on the temperature. Complete conversion of the bicarbonate to carbonate took place in less than three hours at 45° C. at 110 volts and 60 cycles a.c. Of course, at the boiling point the evolution of carbon dioxide due to the partial pressure of this gas would eventually result in the transformation of bicarbonate to carbonate, even in the absence of electrolysis, but continuous boiling of a portion of the bicarbonate solution used in this electrolysis did not free the solution from bicarbonate after six hours' boiling.

The composition of the gas evolved at 110 volts a.c. and 3 amp. per sq. cm. using bright platinum electrodes was: carbon dioxide, 33.3; oxygen, 23.3; hydrogen, 42.0%. The electrolyte was a 2 *N* solution of sodium bicarbonate kept at 18° C. The proportions of carbon dioxide, hydrogen and oxygen in the evolved gases remained practically the same when the current density was raised to 7 amp. per sq. cm., and arcing occurred on the electrodes, but as the amount of platinum black formed on the surface of the electrodes increased, the evolution of carbon dioxide decreased and finally fell to zero, and hydrogen and oxygen only were evolved in diminishing quantities until their evolution finally ceased. The current, however, remained constant even after all evolution of gas had ceased.

In the electrolysis of bicarbonate solutions with iron electrodes the gases were produced at first in the same proportions as when bright platinum electrodes were used, but although the production of carbon dioxide gradually diminished and ceased, hydrogen and oxygen continued to be evolved. The electrodes corroded rapidly with the production of the hydrated oxides of iron.

Decomposition Potential of Carbonate and Bicarbonate Solutions in Respect to the Evolution of Carbon Dioxide

A search of the literature available disclosed nothing definite in regard to ... at which carbon dioxide is evolved from bicarbonate and

carbonate solutions at a platinum electrode. Experiments were carried out to determine the decomposition potential at which carbon dioxide was freed from these ions in d.-c. electrolysis. The presence of carbon dioxide in the evolved gas was detected by passing the anodic gas into barium hydroxide solution. The low partial pressure of carbon dioxide in the solutions used did not interfere with the determination, since the amount of carbon dioxide carried over by the evolved oxygen below the discharge potential of the HCO_3^- was so small as not to interfere with the detection of the carbon dioxide liberated when the discharge potential of the HCO_3^- was reached.

The electrolysis was carried out in a small beaker exposed to the air. The bright platinum electrodes were 12 × 6 mm., and over the anode was a glass tube, immersed to the base of the electrode, from which the evolved anodic gases passed into a capillary tube in the barium hydroxide solution. A plug of cotton wool intercepted the spray that might come over from the anolyte. The barium hydroxide solution was protected from the air by a plug of cotton wool kept moist with sodium hydroxide solution.

A preliminary experiment with sodium hydroxide solution showed that hydrogen and oxygen were freely evolved in the above apparatus at a voltage of 1.9, so it was assumed that from a carbonate or bicarbonate solution any carbon dioxide evolved and not reabsorbed would be carried over with the oxygen from the anode and absorbed by the barium hydroxide solution, forming barium carbonate.

Decomposition Potential of Sodium Carbonate Solutions

Tenth-normal sodium carbonate solution was prepared from freshly fused sodium carbonate dissolved in carbon dioxide-free water. The first indication of carbon dioxide evolution on electrolysis was observed at 3.5 volts with a current density of 0.01 amp. per sq. cm. and at 20° C. At 4.0 volts the evolution of carbon dioxide was rapid. When a little sodium hydroxide solution was added to the electrolyte the voltage necessary to cause evolution of carbon dioxide appeared to be somewhat greater, and when a considerable amount of sodium hydroxide was added the evolution of carbon dioxide was completely inhibited even at voltages up to 110 d.c. and temperatures approaching the boiling point. With the rapid evolution of oxygen under these conditions, and with the top of the anode above the surface of the electrolyte, it was thought that not all of the carbon dioxide would be reabsorbed by the electrolyte.

The above determinations were repeated using various concentrations of sodium carbonate but with the same results. More concentrated solutions gave the same decomposition potential as the more dilute, and the evolution of carbon dioxide was inhibited by the addition of sodium hydroxide even when the voltage was raised to 110 d.c., and the electrodes became hot and arcing occurred.

The addition of sodium sulphate to the carbonate solution even up to the saturation point did not inhibit the evolution of carbon dioxide, so the

explanation of the effect of sodium hydroxide does not lie in the depression of the CO_3^{--} concentration by the excess Na^+ provided by the sodium hydroxide.

Potassium carbonate solutions were prepared and electrolyzed as above and they behaved in a similar manner to the sodium carbonate solutions. The decomposition potential of the potassium carbonate solutions in respect to the evolution of carbon dioxide was found to be 3.5 volts.

Decomposition Potential of Bicarbonate Solutions

Sodium bicarbonate solutions of various concentrations were electrolyzed at room temperature in the apparatus described above. The decomposition potential on bright platinum electrodes was found to be 2.2 volts over a wide range of concentrations. Evolution of carbon dioxide began in mixtures of bicarbonate and carbonate solutions at a voltage of 2.1 to 2.2. The bubbling of air through a $N/10$ sodium bicarbonate solution into barium hydroxide solution gave no appreciable precipitate of barium carbonate. When the same solution was electrolyzed and the evolved oxygen was allowed to carry over the carbon dioxide formed in the electrolysis, a precipitate of barium carbonate was formed when the voltage reached 2.2.

Potassium bicarbonate solutions indicated a decomposition potential at 2.2 volts, when the apparatus described above was used.

Electrode Potentials

The decomposition potential at which carbon dioxide was evolved from sodium bicarbonate solutions was observed to be about 2.2 volts across smooth platinum electrodes, and from sodium carbonate solutions, 3.5 volts. It was thought desirable to measure the single electrode potentials over a considerable range of voltages in the neighborhood of these decomposition potentials in order to compare them with the electrode potentials for solutions of sodium hydroxide over the same range. It is to be noted that the hydrolysis of these salts gives rise to a small concentration of sodium hydroxide in solution, and that consequently the evolution of hydrogen and oxygen occurring simultaneously below, at and above, the decomposition potentials of the bicarbonate and carbonate solutions, would be due to the discharge of H^+ and OH^- ions. Above the decomposition potentials of sodium bicarbonate and sodium carbonate some of the oxygen evolved would be due to the discharge of HCO_3^- and CO_3^{--}.

Smooth platinum electrodes of 1.0 sq. cm. area were cleaned in hot nitric acid, washed in water and ignited. Freshly prepared 2.0 N solutions of sodium hydroxide, sodium bicarbonate and sodium carbonate were used. The electrode potentials were determined by means of a Leeds and Northrup type K potentiometer in conjunction with a calomel electrode, a salt bridge containing the anion under investigation being used. For voltages above the range of the potentiometer a resistance box of 200,000 ohms was inserted across the calomel electrode circuit and the potential across half this resistance read on the potentiometer and multiplied by 2.

The single electrode potentials were determined at regular intervals over the range 1.9 to 4.5 volts, the intervals being determined on a voltmeter always in circuit across the electrodes. The voltmeter readings also served as a rough check on the potentiometer readings, since the sum of the single electrode potentials should approximate that of the voltmeter. Liquid junction potentials were neglected. The current was read on a milliammeter.

Table I contains the results of the observations recorded to the accuracy of reproducibility. No readings were recorded until the current had been flowing for at least 10 min.

TABLE I

CURRENT DENSITY AND SINGLE ELECTRODE POTENTIALS IN THE d.-c. ELECTROLYSIS OF 2 *N* SOLUTIONS OF SODIUM HYDROXIDE, SODIUM CARBONATE AND SODIUM BICARBONATE

Voltmeter reading, volts	NaOH			Na_2CO_3			$NaHCO_3$		
	Milliamps. per cm.²	Cathode volts	Anode volts	Milliamps. per cm.²	Cathode volts	Anode volts	Milliamps. per cm.²	Cathode volts	Anode volts
1.9	7.5	+0.86	−1.03	5.5	+0.76	−1.12	6.0	+0.42	−1.41
2.0	7.5	+0.93	−1.04	6.0	+0.84	−1.17	7.5	+0.50	−1.43
2.2	10.0	+1.05	−1.11	7.5	+0.93	−1.31	7.5	+0.64	−1.50
2.4	12.5	+1.16	−1.17	10.0	+1.01	−1.41	8.0	+0.74	−1.59
2.6	28.0	+1.22	−1.27	14.0	+1.07	−1.52	10.0	+0.85	−1.67
3.0	40.0	+1.30	−1.61	17.0	+1.13	−1.84	12.5	+1.03	−1.89
3.5	75.0	+1.37	−1.97	20.0	+1.31	−2.08	17.5	+1.14	−2.25
4.0	200.0	+1.50	−2.38	42.0	+1.48	−2.52	30.0	+1.26	−2.71
4.5	250.0	+1.66	−2.78	58.0	+1.62	−2.86	45.0	+1.39	−3.20

The evolution of visible gas bubbles on the electrodes was not observed at a voltage less than 1.9 and, as will be seen from the current density-electrode voltage curves, Fig. 2, this is about the lower limit for any significant changes of voltage with current density.

Fig. 1 is a graph of the electrode potentials corresponding to the impressed voltage across the electrodes, as plotted from the data of Table I.

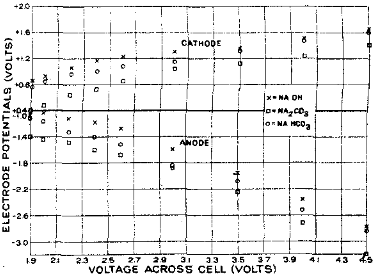

FIG. 1. *Graph showing change of single electrode potential with impressed voltage for sodium hydroxide, sodium carbonate and sodium bicarbonate solutions.*

It will be observed from Fig. 1 that for the same total electrode voltage the cathode potential during electrolysis is always more positive in the sodium hydroxide solution than in the sodium carbonate solution, and even more so than in the solution of sodium bicarbonate. The anode potential on the other

hand is always less negative in the sodium hydroxide solution than in the sodium carbonate solution and considerably less than in the sodium bicarbonate solution. The increase in the potential at each electrode with increase in the impressed voltage is practically the same throughout, although it will be noted that the negative potential increases much more rapidly than does the positive potential. This is no doubt due to the increased polarization of the negative electrode when oxygen is being evolved at increasing current densities as compared with the polarization due to hydrogen at the cathode (1, 2, 3, 4). The effect of carbon dioxide evolution on polarization must be very little, since no marked differences in the relation of the electrode potentials occur at the voltages corresponding to the observed beginning of evolution of this gas (-1.5 to -1.6 volts discharge potential for HCO_3^- and -1.9 to -2.0 volts discharge potential for CO_3^{--}).

The order and magnitude of the differences in potential of the electrodes during electrolysis in the three solutions are in accordance with the (H^+) and (OH^-) concentrations, as would be expected. The sodium hydroxide solution, being more strongly basic than the sodium carbonate, a higher positive potential is required to discharge H^+ and a lower negative potential to discharge OH^- than for either of the salt solutions. Similarly hydrolysis of sodium carbonate produces a higher concentration of OH^- than does the hydrolysis of sodium bicarbonate, and consequently a lower negative potential is required to discharge OH^- and higher positive potential to discharge H^+.

Current Density and Electrode Potential

When the electrode potentials are plotted against current density, as in Fig. 2, it is observed that the cathode potentials for the three solutions do not differ very markedly for the same current density. At current densities higher than 20 milliamp./cm.[2] the voltage rises more rapidly for the sodium hydroxide solution and least rapidly for the sodium carbonate solution. On the other hand, there is a marked difference in the electrode potentials of the anode at the same current density, that in the sodium hydroxide solution rising abruptly with current densities greater than 10 milliamp./cm.[2], as compared with those in the sodium carbonate and sodium bicarbonate solutions. The order of the increasing electrode voltages is the reverse of the OH^- concentration of the electrolyte. At lower current densities there is also a greater spread in the anode potentials as compared to the cathode potentials.

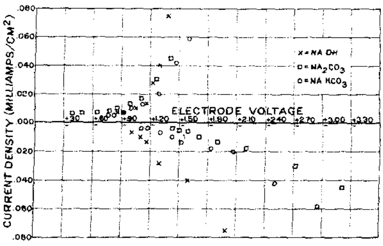

FIG. 2. *Graph showing relation of electrode potentials to increasing current density for sodium hydroxide, sodium carbonate and sodium bicarbonate solutions.*

A relatively greater change in potential with increase in current density takes place at the anode in the neighborhood of the discharge potentials of HCO_3^-, −1.45 to −1.50 volts, and of CO_3^{--}, −1.90 to −1.95 volts, particularly in the case of sodium carbonate. At higher voltages the curves approximate normal decomposition potential curves, that is, the increase in electrode voltage drops off rapidly as compared with the increase in current density.

Discharge Potentials

The discharge potentials of HCO_3^- and CO_3^{--} correspond to the anode voltage at which carbon dioxide was detected in the anode gas discharge, and those of OH^-, to the anode voltage at which oxygen gas first appeared on the electrode. The total electrode voltages corresponding to these potentials were approximately 2.2, 3.5 and 1.9 volts respectively. The electrochemical relations for the discharge potentials may be expressed as in Table II.

TABLE II

Ion	Discharge potential, volts	Electrochemical equation
OH^-	−1.00	$4OH^- - 4e = 2H_2O + O_2$
HCO_3^-	−1.45 to −1.50	$4HCO_3^- - 4e = 2H_2O + 4CO_2 + O_2$
CO_3^{--}	−1.90 to −1.95	$2CO_3^{--} - 2e = 2CO_2 + O_2$

It might be expected that evolution of carbon dioxide would be continuous at all voltages greater than the discharge potential of HCO_3^- even in sodium carbonate solutions, since sodium carbonate produces HCO_3^- by hydrolysis. No such evolution however was observed in sodium carbonate solutions until a decomposition potential of 3.5 volts was reached. On consideration however it is seen that if carbon dioxide were evolved owing to the discharge of HCO_3^- in sodium carbonate solution, it would react with the free alkali formed by hydrolysis to reform sodium carbonate in order to preserve the ionic equilibrium of hydrolysis.

Discussion

No great degree of accuracy can be claimed for the values obtained for the discharge potentials of HCO_3^- and CO_3^{--} ions, because the point of discharge was determined by the appearance of carbon dioxide in the evolved anodic gas. The partial pressure of carbon dioxide from the solutions was an uncontrollable variable affected by the composition of the solution and the temperature. It is to be expected that the decomposition voltages reported will be, if anything, a little high, since the method of detecting this voltage depended on the evolution of carbon dioxide together with that of the oxygen at the anode, and electrolytic decomposition of bicarbonate or carbonate could be recognized only when a distinct precipitate of barium carbonate, which increased visibly on increasing the voltage, was obtained on passing the evolved gas through barium hydroxide solution.

No irregular behavior of the single electrode potentials occurs over the range studied. The effect of the bicarbonate ions, carbonate ions and evolved carbon dioxide on the platinum anode is apparently negligible, as the voltages observed are in conformity with what would be expected from the polarizing effect of oxygen alone and the mutually compensating effect of the H^+ and OH^- ion concentration on the discharge potentials of the cathode and anode.

The inhibiting effect of sodium hydroxide on the evolution of carbon dioxide is probably only apparent and may be explained by the reabsorption of the carbon dioxide by the sodium hydroxide before it reached the barium hydroxide.

Practically, the decomposition potential of water, when platinum electrodes are used, is raised appreciably by the presence of carbonate and bicarbonate, and thus the electrical energy absorption is increased. It would be interesting to determine what this increase actually is in the case of iron and nickel electrodes, and to know whether the sodium hydroxide used technically for the electrolysis of water is sufficiently free from carbonate to obviate any increase in the decomposition potential.

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RAPID DETERMINATION OF MOISTURE IN GRAIN

III. CALIBRATION AND COMPARISON OF ELECTRICAL MOISTURE METERS WITH VACUUM OVEN FOR AMBER DURUM WHEAT, BARLEY AND OATS[1]

By W. H. Cook,[2] J. W. Hopkins[3] and W. F. Geddes[4]

Abstract

The previous study has been extended to include durum wheat, barley and oats. The hand-operated Tag-Heppenstall meter was found to be unsatisfactory with these grains, as they would not feed into the roller electrodes in a suitable manner. The Burton-Pitt gave erratic results with these grains and it was only possible to calibrate this meter over a limited moisture range, and even over this range it was more inaccurate than the other meters. Qualitatively the calibration curves for these three grains, in the Limbrick and motor Tag-Heppenstall, were similar to those previously obtained with hard red spring wheat. The actual resistance and the slope of the curves were, however, somewhat different for the different grains. The standard error of prediction shows that the motor-operated Tag-Heppenstall was the most accurate meter for use with durum wheat and barley, while the Limbrick was superior with oats. With the limited number of samples available it was impossible to detect any significant difference between the temperature coefficients, in any particular meter, of the different grains. When converted to a moisture basis the correction factors were practically the same as for hard red spring wheat.

The results from the entire investigation show that the Brown-Duvel method is more accurate than the 130° C. air oven method with all grains studied. The motor-operated Tag-Heppenstall meter is as accurate as the Brown-Duvel with hard red spring wheat, over the moisture range 11.0 to 17.0%, and is superior to the air oven method over this limited range. Otherwise the rapid analytical methods are more accurate than any of the moisture meters tested with any of the grains. The meters fall in the following order of decreasing accuracy over the moisture range 11.0 to 17.0%:—with hard red spring wheat; motor Tag-Heppenstall, Limbrick, hand Tag-Heppenstall, Burton-Pitt and Davies: with durum wheat and barley; motor Tag-Heppenstall, Limbrick and Burton-Pitt: and with oats; Limbrick, Burton-Pitt and motor Tag-Heppenstall. Where a meter is not mentioned no tests were made, the instrument having been omitted because it gave no promise of practical utility.

1. Introduction

The scope and plan of this investigation have already been given in earlier papers of this series (*1, 2*). In the present paper, the results obtained with

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the Burton-Pitt, Limbrick, and hand- and motor-operated Tag-Heppenstall meters upon amber durum wheat, barley and oats, are compared with those obtained with the vacuum oven. Since the Davies meter proved quite unsuitable for hard red spring wheat (2), it was not included in the present study.

Approximately 50 samples of durum wheat and 75 of oats and of barley were employed. These were the market run of grades during the collection period but were selected with respect to moisture content so as to give a fairly uniform distribution throughout the moisture range. Since it was impossible to secure naturally damp (over 17.0% moisture) durum samples, it was necessary to prepare these. Owing to the possibility of artificially tempered grain behaving differently from naturally damp samples (2) even when allowed to stand a month after tempering, these samples were dampened by exposing them to a high relative humidity at room temperature, and then allowing them to dry down to the observed moisture content. Whether or not they behaved differently from naturally damp grain is not known as there were no naturally damp samples available for comparison.

2. Experimental Details

As in the previous experiments (2) with hard red spring wheat, the instruments were, with the exceptions noted below, operated in accordance with the manufacturers' instructions, and the sensitivities, settings and operation of the instruments were identical with those previously described. The Burton-Pitt cell would not hold 100 gm. of barley or oats, and the maximum quantity of these grains that could be employed was 80 and 60 gm. respectively. As the Limbrick cell is filled by volume no modification of the technique was required. None of these grains would feed into the rollers of the hand-operated Tag-Heppenstall meter satisfactorily at setting No. 3, which corresponded most nearly to the spacing of 40/1000 in. recommended by the manufacturers, and No. 4 and 5 were consequently tried. A few durum samples at No. 5 setting excepted, the feed was still too irregular to permit of accurate readings of the microammeter and the use of this meter with these grains was finally abandoned. The motor Tag-Heppenstall appeared to operate satisfactorily at the recommended spacing of 40/1000 in. with durum wheat and barley, but oats sometimes failed to feed through unless the undriven roller was rotated a revolution or so by hand. The microammeter readings, however, remained reasonably constant while the rolls were in operation. A roll spacing of 50/1000 in. was also tested with oats.

As before, the meter tests were made in a room conditioned as to temperature and humidity. The general reliability tests were made at 76° F., instead of 72° F. as in the hard red spring wheat studies, since it was found possible to maintain the higher temperature within narrower limits. Even with this precaution, the temperature occasionally rose 1–2° F. and the readings obtained were therefore corrected to the 76° F. basis before making

the statistical analyses. The relative humidity employed was 40% ± 2%, as before, and the corrections for humidity variations have already been shown in the previous experiments (2) to be too small to affect the results.

3. Effect of Temperature

Owing to the difficulty of determining accurately the temperature coefficient of the grain alone, no attempt was made to measure it with the limited number of samples available. The effect of humidity was not investigated as it has already been shown to be small, and the humidity coefficients already determined for wheat can also be regarded as applicable to these grains, since the influence of humidity must be attrihuted to its effect on the instruments rather than on the grain, which was kept in sealed tins until the moment of testing.

The effect of temperature was studied, using 68, 72, 76, 80, 84 and 88° F. $\pm\frac{1}{2}$° F. and a relative humidity of 40% ± 2%. Four large samples of durum wheat, 10 of barley, and 12 of oats were subdivided as before for the various tests, these constituting all the large samples available for this study. The grades and moisture contents of these samples are given in Table I.

TABLE I

GRADE AND VACUUM OVEN MOISTURE CONTENT OF DURUM WHEAT, BARLEY AND OAT SAMPLES USED IN STUDYING EFFECT OF TEMPERATURE ON MOISTURE METER RESULTS

Grade*	Percentage moisture by vacuum oven						
	Trial 1	*Trial 2*	*Trial 3*	*Trial 4*	*Trial 5*	*Trial 6*	Average
			Amber durum wheat				
1 C.W. Amber durum	14.46	14.49	14.40	14.53	14.40	14.53	14.47
Tf. 2 C.W. Amber durum	16.32	16.20	16.06	16.07	15.98	16.26	16.15
Tf. 3 C.W. Amber durum	16.19	16.10	16.03	—	15.98	16.20	16.10
Tf. 2 C.W. Amber durum	16.93	17.06	16.92	17.03	16.73	16.78	16.91
			Barley				
3 Ex. C.W. Six-row	14.41	14.34	14.46	14.45	14.40	14.40	14.41
3 Ex. C.W. Six-row	13.72	13.28	13.34	13.32	13.28	13.30	13.37
3 Ex. C.W. Six-row	14.66	14.59	14.70	14.56	—	14.62	14.63
Tf. 3 C.W.	17.14	17.00	17.00	17.10	16.91	17.00	17.03
Tf. 3 C.W.	14.92	14.96	14.78	14.94	14.75	14.90	14.88
Tf. 3 C.W.	16.54	16.48	16.34	16.50	16.46	16.48	16.47
Tf. 3 Ex. C.W. Six-row	17.72	17.80	17.78	17.78	17.76	17.66	17.75
Dp. 3 C.W.	22.04	21.76	21.70	21.82	21.82	21.90	21.84
Dp. 3 C.W.	—	19.64	19.56	19.66	19.52	19.76	19.63
Dp. 3 Ex. C.W. Two-row	22.35	22.37	22.12	22.25	22.20	22.44	22.29
			Oats				
3 C.W.	13.11	13.10	13.26	13.06	13.12	13.08	13.12
3 C.W.	14.39	14.52	14.40	14.35	14.42	14.37	14.41
1 Feed	14.57	14.61	14.79	14.66	14.60	14.60	14.64
Tf. 2 C.W.	15.34	15.32	15.42	15.28	15.32	15.26	15.32
Tf. 2 Feed	17.45	17.37	17.54	17.20	17.47	17.44	17.41
Dp. 3 C.W.	17.62	17.63	17.72	17.56	17.73	17.62	17.65
Dp. 1 Feed	17.60	17.57	17.76	17.54	17.57	17.56	17.60
Dp. 3 C.W.	19.61	19.62	19.68	19.57	19.68	19.64	19.63
Dp. 3 C.W.	18.04	17.86	18.14	17.11	17.98	18.12	17.88
Dp. 1 Feed	19.30	19.30	18.69	19.25	19.30	19.34	19.20
Dp. Ex. 1 Feed	22.07	22.08	22.08	22.10	22.06	21.94	22.06
Dp. Ex. 1 Feed	22.48	22.55	22.60	22.51	22.59	22.54	22.55

* *Tf.* = *tough.* *Dp.* = *damp.*

The results obtained with the three meters are plotted in Fig. 1 for both durum wheat and barley, and in Fig. 2 for oats. With the Burton-Pitt, the meter readings are plotted against the temperature, but with the resistance type instruments the logarithm of the resistance is employed since this quantity is proportional to the moisture content over the greater part of the moisture range. As the resistances measured by the Limbrick were much higher than those encountered in the Tag-Heppenstall, the megohm and 1000 ohms respectively were selected as units of resistance.

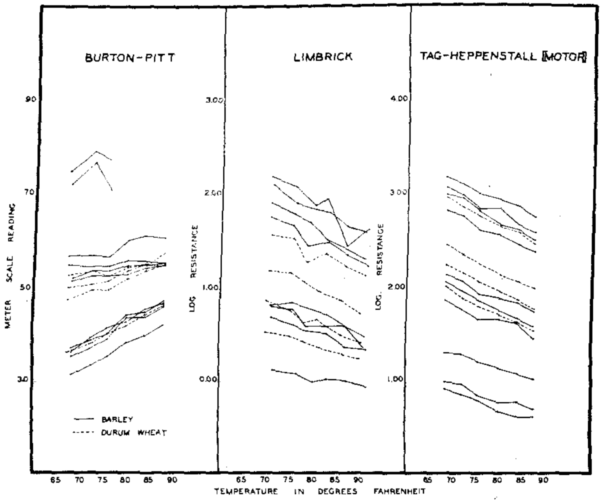

FIG. 1. *Effect of temperature of grain-instrument system on meter reading. (Durum wheat and barley).*

It is evident from Figs. 1 and 2 that the Burton-Pitt gave erratic results at deflections greater than about 55 scale divisions, as it did with hard red spring wheat. The variability observed with any one sample in the other two meters can doubtless be attributed, as before, to experimental and sampling errors.

In order to determine the temperature coefficient, a straight line was fitted to the points obtained with each sample, by the method of Least Squares. The equations were then differentiated to obtain the temperature coefficients, which are plotted against the observed meter reading or logarithm of the resistance at 76° F. (79° F. for the Limbrick) in Fig. 3. With the limited number of samples tested, it was impossible to demonstrate any difference

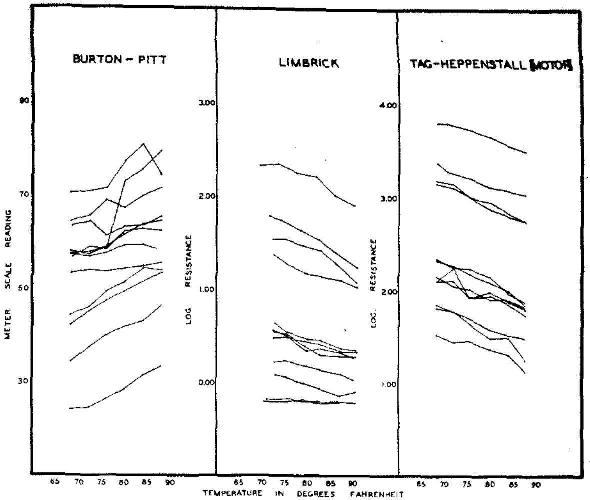

FIG. 2. *Effect of temperature of grain-instrument system on meter reading. (Oats).*

TABLE II

TEMPERATURE COEFFICIENT OF GRAIN-INSTRUMENT SYSTEM
(Grain and meter at same temperature)

Meter	Meter range	Temp. coefficient per ° F.	Standard error	Unit of measurement
Burton-Pitt	20 – 70	0.30	0.05	Meter scale division
Limbrick	over 1.00 1.00 – 0.50 0.50 – 0.00 under 0.00	−0.0248 −0.0168 −0.0144 −0.0056	0.0015 0.0014 0.0009 0.0028	Logarithm of resistance in megohms
Tag-Heppenstall (motor)	4.00 – 0.50	−0.0214	0.0007	Logarithm of resistance in 1000's of ohms

between the temperature coefficients for the three grains. It is evident that the Limbrick meter is the only one in which there is a systematic variation in the coefficient with observed resistance (moisture content), the Burton-Pitt instrument giving erratic results, and the coefficients for the motor Tag-

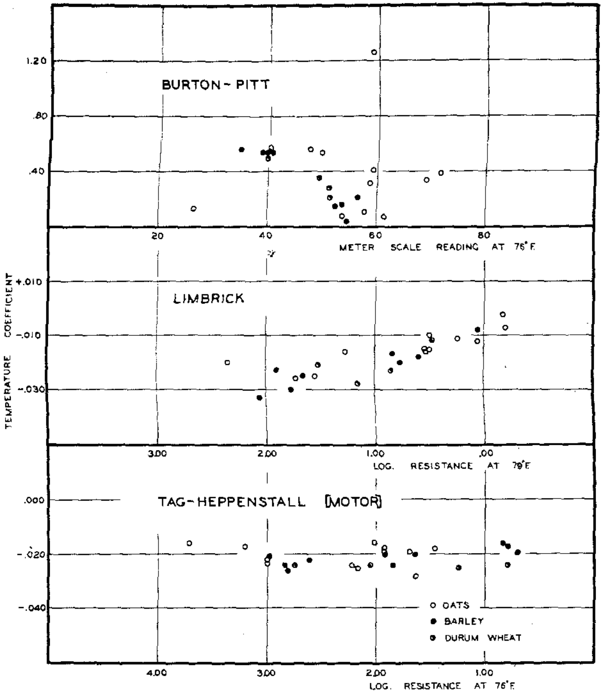

Fig. 3. *Variation of temperature coefficient of grain-instrument system with moisture content. (Durum wheat, barley and oats).*

Heppenstall being practically the same throughout the range. The values used for correcting the results obtained in the general reliability series for small variations in temperature are given in Table II. Owing to the variability of the coefficients obtained with the Burton-Pitt an average of all the coefficients was used.

4. Calibration and Accuracy of Meters

The meters were tested in a conditioned room as before and the readings were corrected for the small temperature fluctuations which occurred. A

standard temperature of 76° F. was used for the Burton-Pitt and motor Tag-Heppenstall meters, and a temperature of 79° F. for the Limbrick to allow for the heating of the grain in the preparatory grinding.

The calculations necessary to calibrate the meters with all three grains, and to determine the degree of accuracy attained, were made in exactly the same manner as those already described in the study with hard red spring wheat. Qualitatively, the calibration curves were all found to be similar to those previously obtained with hard red spring wheat, the resistance instruments indicating a linear relation between moisture content and logarithmic resistance up to about 17.0% moisture, and a curvilinear relation at higher moisture contents, where successive increments of moisture resulted in a progressively smaller diminution in resistance. Quantitatively, however, some differences were exhibited by the various grains.

Burton-Pitt Tester

Apart from reduction of the weight of barley and oats to an amount which the test-cell would accommodate, these experiments were conducted at the same sensitivity and in the same manner as those with hard red spring wheat already described. The data are shown in Fig. 4 where the moisture content by the vacuum oven is plotted against the meter scale reading. It is evident from the scattered points that the meter is not capable of great precision with any of the three grains even at low moisture content, and at high moisture contents it gives exceedingly erratic results. In consequence, curves were fitted over the lower moisture range only, and the upper limit of this range varied with the different grains according to the quality of the results. Within the range of calibration the curves are all of the same type as those obtained with hard red spring wheat, namely, parabolic with a tendency toward greater deflections with successive increments of moisture.

The experimental error, calibration equations, and errors of prediction applicable to the different grains over the range covered by the curves, are given in Table III. The experimental errors are practically the same, in all

TABLE III

Experimental error, calibration equations, and observed and net standard error of prediction of vacuum oven moisture content of durum wheat, barley and oats by the Burton-Pitt moisture tester

Grain	Moisture range, %	Experimental error (standard error of duplicates), moisture %	Calibration equation*	Standard error of prediction	
				Observed, moisture %	Net, moisture %
Amber durum wheat	11.5 – 18.5	0.06	$M_V = 7.56 + 0.199x - 0.000718x^2$	0.46	0.44
Barley	11.0 – 16.0	0.09	$M_V = 4.42 + 0.401x - 0.00340x^2$	0.84	0.83
Oats	12.5 – 15.5	0.05	$M_V = 8.23 + 0.244x - 0.00186x^2$	0.41	0.39

Standard errors apply to mean of duplicates.
**M_V = moisture content as determined by vacuum oven.*
x = meter scale reading.

cases, as those obtained with hard red spring wheat. The errors of prediction increase in the order: oats, durum wheat and barley, but, since the calibrated range was different in each case and was more or less arbitrarily determined,

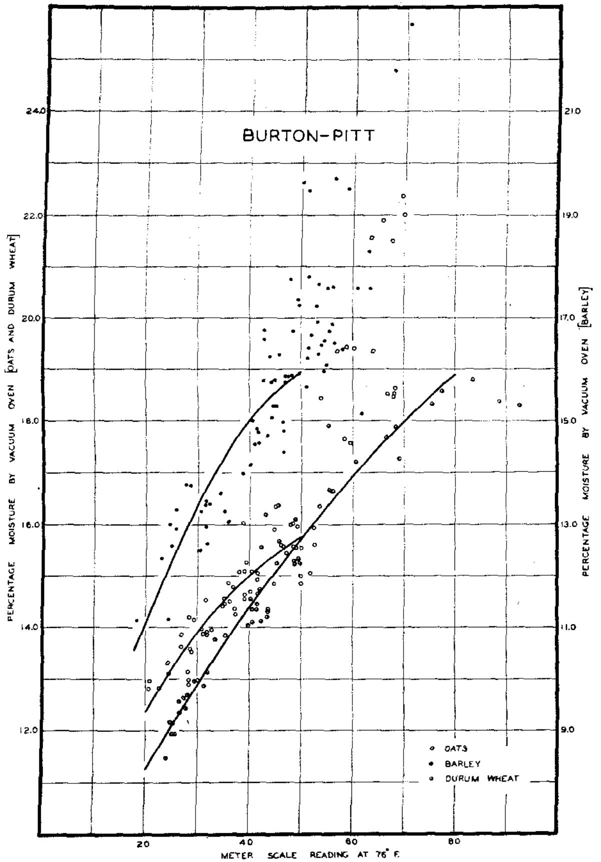

FIG. 4. *Relation between moisture content of durum wheat, barley and oats, and Burton-Pitt meter reading.*

the curves are not strictly comparable. Examination of Fig. 4 shows that, in general, this meter is less accurate with oats and barley than with durum wheat. Since the lowest error of prediction obtained, even in the lower moisture range, was 0.39% moisture, it is obvious that this meter is incapable of giving a precise estimate of the moisture content of any of the grains tested.

Limbrick Moisture Tester

The moisture content of the samples of all three grains is plotted against the logarithm of the resistance, in megohms, in the Limbrick at 79° F. in Fig. 5. Two curves were fitted to the points for each grain, one representing the linear relationship at moisture contents below about 17.0% and the other the parabolic relationship at higher moisture contents, where successive increments of moisture result in a progressively smaller diminution in resistance. Owing to the relatively small number of samples, the extremes of the two curves do not coincide. In such instances a dotted tangent indicates the probable approximate course of the actual calibration curve.

In this meter, for which the grain is ground, the two covered grains yield practically identical calibration curves, although the individual results with oats are the more erratic. Durum wheat on the other hand yields a significantly different calibration, a given change in moisture content causing a greater change in resistance. This resembles more closely the calibration deduced for hard red spring wheat. Statistical comparisons were made between the calibration equations in the "straight" and "tough" moisture range for hard red spring wheat and durum wheat, and for barley and oats. It was found that the average resistance of durum wheat was lower than that of hard red spring wheat, and also that the change in resistance per unit change in moisture was slightly greater. Similar calculations showed that oats had a slightly higher resistance than barley at a given moisture content, but that there was no significant difference in the slope of the two calibration curves. It is probable that the higher resistance of oats can be attributed to the smaller amount employed, the weight per unit volume of ground oats being less than that of ground barley.

The experimental error, calibration equations and errors of prediction applicable to each grain are given in Table IV. The experimental errors with durum wheat and barley are somewhat smaller than those encountered with hard red spring wheat. That with oats, however, is somewhat larger, especially in the "damp" range. This large experimental error can doubtless be attributed to the light chaffy character of ground oats. It is difficult to fill and strike off the small measuring cup precisely with such material, and in addition the resistance reading is dependent on the material falling between the prongs and walls of the test cell in a uniform manner in different trials, a condition which probably is not attained to the same extent with oats as with the other grains. In spite of this large experimental error, however, the error of prediction with oats is of the same order as that obtained with wheat. This suggests that although the meter is not particularly accurate in measuring

the resistance of ground oats, the relationship between resistance and moisture content is the predominating factor governing the accuracy of the meter.

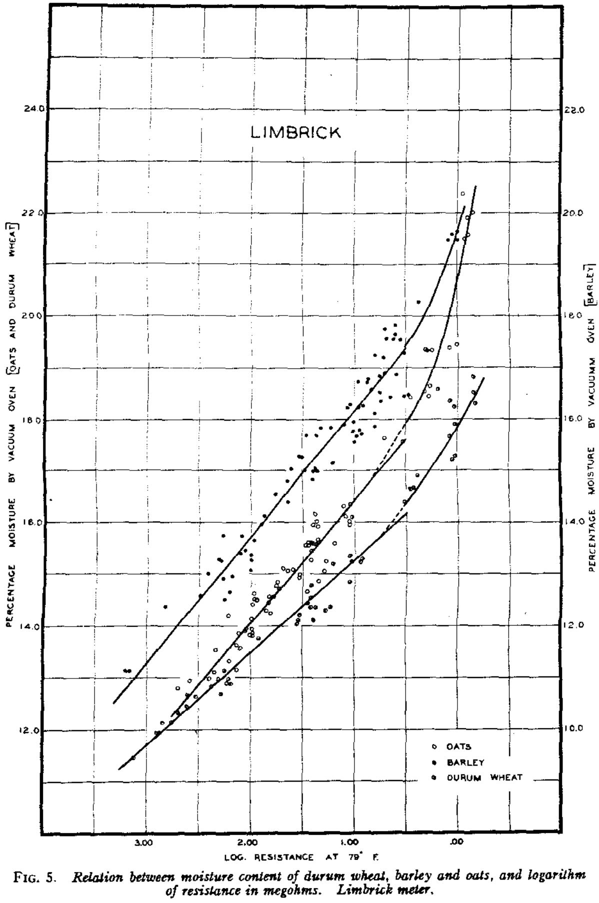

FIG. 5. *Relation between moisture content of durum wheat, barley and oats, and logarithm of resistance in megohms. Limbrick meter.*

It is evident from the error of prediction that with these grains, as with hard red spring wheat, this meter is, in general, capable of greater accuracy in the moisture range below, than in the moisture range above 17.0%. In this lower moisture range it is less accurate with oats than with durum wheat and is still less accurate with barley. Above 17.0% moisture this meter is somewhat less accurate with oats than with barley.

TABLE IV

EXPERIMENTAL ERROR, CALIBRATION EQUATIONS, AND OBSERVED AND NET STANDARD ERROR OF PREDICTION OF VACUUM OVEN MOISTURE CONTENT OF DURUM WHEAT, BARLEY AND OATS BY THE LIMBRICK MOISTURE TESTER

Grain	Moisture range, %	Experimental error (standard error of duplicates), moisture %	Calibration equation*	Standard error of prediction	
				Observed, moisture %	Net, moisture %
Amber durum wheat	11.5 – 16.0	0.04	$M_V = 17.07 - 1.79 \log R$	0.33	0.29
	16.0 – 19.0	0.05	$M_V = 17.84 - 3.52 \log R + 1.16 (\log R)^2$	0.34	0.30
Barley	11.0 – 17.5	0.07	$M_V = 18.61 - 2.44 \log R$	0.44	0.42
	17.5 – 20.0	0.06	$M_V = 19.65 - 6.62 \log R + 4.28 (\log R)^2$	0.52	0.50
Oats	12.5 – 17.0	0.12	$M_V = 18.78 - 2.38 \log R$	0.32	0.30
	17.0 – 22.0	0.19	$M_V = 20.74 - 8.88 \log R + 6.56 (\log R)^2$	0.58	0.57

Standard errors apply to mean of duplicates.
$^*M_V$ = *moisture content as determined by vacuum oven.*
R = *resistance in megohms.*

MOTOR-OPERATED TAG-HEPPENSTALL METER

This Tag-Heppenstall meter was operated at a roll spacing of 40/1000 in. with all three grains, as specified by the manufacturers. It was found, however, that oats did not feed into the rollers satisfactorily at this spacing, the undriven roller frequently stopping, requiring to be rotated through a revolution or two by hand. Furthermore, the microammeter needle seemed to fluctuate more than in the tests with other grains. A roll spacing of 50/1000 in. was also tested, but this gave no significant increase in accuracy.

In Fig. 6, the moisture content of the samples of all three grains is plotted against the logarithm of the resistance in 1000's of ohms, at a temperature of 76° F. and a roll spacing of 40/1000 in. As before, two curves were fitted to the points for each grain, one representing the linear relationship at moisture contents below about 17.5%, and the other the parabolic relationship at higher moisture contents.

In this meter, for which the grain is not ground, the change in resistance per unit change in moisture is again practically the same for both oats and barley, but the actual resistance at a given moisture content is much higher for oats. In the Limbrick, for which the grain is ground, oats exhibited only a slightly higher resistance than barley. This suggests that the resistance of

one of these grains is altered appreciably by grinding, but since the resistances of the whole and ground material were determined in two different instruments

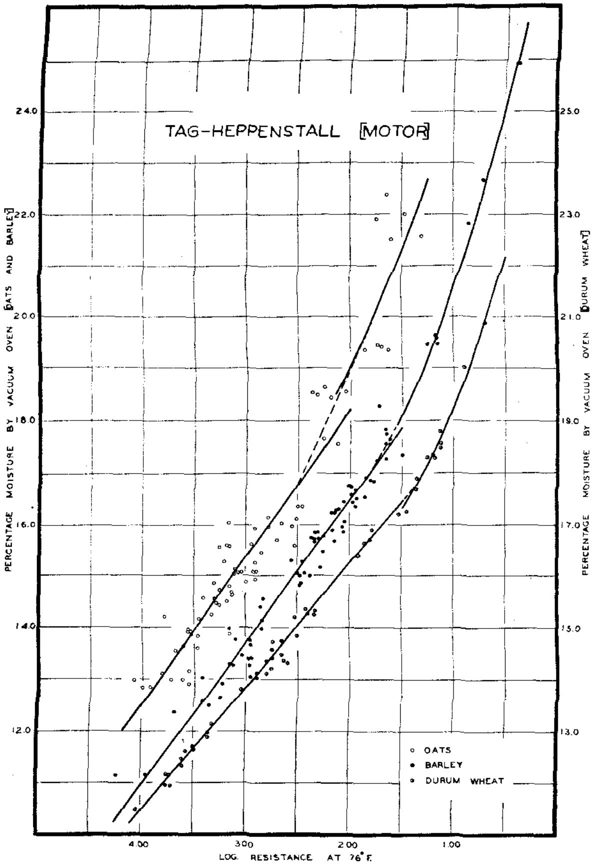

FIG. 6. *Relation between moisture content of durum wheat, barley and oats, and logarithm of resistance in 1000's of ohms. Motor-operated Tag-Heppenstall meter.*

it cannot be said which one was affected. On the other hand this apparent change in the resistance may be attributable to some peculiarity of the instruments, especially since difficulty was experienced in feeding oats into the roller electrodes of the Tag-Heppenstall meter satisfactorily. The calibration curves for oats and barley did not differ significantly in slope over the moisture range 11.0 to 17.0%.

It is evident from Fig. 6 that the durum wheat shows a slightly greater change in resistance, for a given change in moisture content, than the two covered grains. Comparisons were made between the slope and actual resistances of the calibration curves obtained with durum and hard red spring wheat. It was found that durum wheat has a significantly lower resistance than hard red spring wheat, but the difference in the slopes did not significantly exceed the standard error. These two wheats, although differing in actual resistance, seem to show the same relationship between increments of moisture and resistance, a fact which may be helpful in preparing calibration charts for them.

The experimental error, calibration equations, and errors of prediction applicable to each grain are given in Table V. The experimental error with durum wheat is practically the same as that previously obtained with hard red spring wheat, somewhat larger with barley, and still larger with oats. The standard error of prediction is least with durum wheat, intermediate with barley, and extremely high with oats, this meter being inferior to the Limbrick with this grain. This result cannot be attributed to the experimental errors, for although these fall in the same order for the different grains, they are in all cases small compared with the error of prediction. The magnitude of the prediction errors shows definitely that the instrument

TABLE V

EXPERIMENTAL ERROR, CALIBRATION EQUATIONS, AND OBSERVED AND NET STANDARD ERROR OF PREDICTION OF VACUUM OVEN MOISTURE CONTENT OF DURUM WHEAT, BARLEY AND OATS BY THE MOTOR-OPERATED TAG-HEPPENSTALL MOISTURE METER

Grain	Moisture range, %	Experimental error (standard error of duplicates), moisture %	Calibration equation*	Standard error of prediction	
				Observed, moisture %	Net, moisture %
Amber durum wheat	11.5 – 17.5	0.02	$M_V = 21.00 - 2.38 \log R$	0.20	0.13
	17.5 – 22.0	0.04	$M_V = 26.05 - 8.81 \log R + 1.99 (\log R)^2$	0.18	0.10
Barley	11.0 – 17.5	0.04	$M_V = 21.96 - 2.75 \log R$	0.32	0.29
	17.5 – 25.0	0.06	$M_V = 28.66 - 10.38 \log R + 2.24 (\log R)^2$	0.37	0.34
Oats	12.5 – 17.5	0.07	$M_V = 23.90 - 2.85 \log R$	0.56	0.55
	17.5 – 22.5	0.13	$M_V = 32.58 - 9.84 \log R + 1.55 (\log R)^2$	1.01	1.00

Standard errors apply to mean of duplicates.
*M_V = *moisture content as determined by vacuum oven.*
R = *resistance in 1000's of ohms.*

is much less reliable with the covered grains than with wheat. It seems probable that the error of prediction could be lowered by grinding the covered grains, especially oats, since the Limbrick, for which the grain is ground, gave a lower error of prediction with oats even though the experimental error was relatively high.

Day-to-day Variations

As explained in the previous paper (2) three large samples of each of the grains were tested daily in all meters to determine to what extent the results were affected by daily fluctuations. Owing to the small number of samples included in the general reliability series, the test with any one grain usually did not extend over more than two or three days, and the results obtained from the above large samples showed no evidence of daily fluctuations within this limited period. Calculation of the variance and covariance of the results in the general reliability tests, around the daily means as well as around the general means, likewise gave no evidence of daily variations, except to a slight extent in the barley experiments with the Burton-Pitt.

5. Temperature Corrections in Terms of Moisture

The temperature coefficients, given earlier in Table II, were obtained by pooling the results from the limited number of samples of the individual grains, and while adequate for correcting the results of the general reliability tests for the small variations which occurred, are not recommended for general use. They are, however, judged to be sufficiently accurate for "straight" and "tough" samples of oats and barley in the two resistance instruments to permit temperature corrections to be made over a range of $\pm 10°$ F. Correction values, showing the percentage moisture to be added or subtracted for each degree F. above or below the temperature at which the calibration curve is applicable were therefore computed for these grains and meters as previously described (2) and are

TABLE VI

Temperature corrections in terms of per cent moisture
(Grain and meter at same temperature)

Meter	Standard temperature, °F.	Correction in terms of per cent moisture for each degree F. above or below the standard temperature	
		Straight	*Tough*
Barley			
Limbrick	79	0.06	0.05
Tag-Heppenstall (motor)	76	0.06	0.06
Oats			
Limbrick	79	0.06	0.05
Tag-Heppenstall (motor)	76	0.06	0.06

Note: *Corrections are to be* added *if the temperature is below the standard, and* subtracted *if above the standard.*

listed in Table VI. The corrections for the two meters differ slightly, but the corresponding values are practically the same as those for hard red spring wheat (*cf.* (2) Table X). This suggests that the same correction may be applicable to all grains.

7. General Summary and Conclusions

As a matter of convenience, a brief summary of the results obtained from the entire investigation is given here. The result of an analysis of variance on the vacuum oven data has already been summarized in the first paper of this series ((1) Table III). The sampling error proved to be the greatest single source of variation. Day-to-day variations in the results also occurred, and the two vacuum ovens, although of identical construction, were found to give results differing on the average by 0.07% moisture. It is probable that the average differences between ovens and the day-to-day variations are to some extent different measurements of the same effect.

The results obtained with the 130° C. air oven, and the Brown-Duvel have also been summarized ((1) Table VIII). It is evident from these that the Wiley mill was superior to the Hobart grinder for use in conjunction with the air oven method. The Brown-Duvel method proved, under the conditions of these experiments, to be superior to the 130° C. air oven, even when the Wiley mill was employed. Both methods, however, underestimate the moisture content of all the grains studied, but this systematic error may be eliminated by means of the linear correction equations given in the first paper ((1) Tables IV, VI and VII).

The experimental error, and the net standard error of prediction of vacuum oven moisture content for all meters calibrated and all grains tested, are given in Table VII. It is evident that the experimental error, as in the rapid analytical methods, is in all cases small compared with the standard error of prediction, indicating that the meters are capable of measuring the resistance, or other electrical quantity, accurately enough, but that this is affected by some physical property of the grain other than moisture content. This property may be inherent in the grain or it may be induced by the instrument, *e.g.*, by polarization. The meters are arranged in the table in order of decreasing accuracy, as determined by the standard error of prediction in the moisture range 11.0 to 17.0%, which is most important commercially, for each of the grains tested. It is evident that the motor-operated Tag-Heppenstall is the most accurate of the meters for all grains excepting oats, for which the Limbrick proved to be best, possibly because it employs ground grain. The Burton-Pitt and Davies instruments are judged of little practical value.

The results by all meters were found to be appreciably affected by fluctuations in temperature, and the temperature coefficient of wheat alone was found to differ appreciably from that of the grain-instrument system in the Burton-Pitt and Limbrick testers, as shown in the second paper of the series ((2) Table X), suggesting a significant instrumental temperature coefficient.

The meters were also all influenced to some extent by humidity conditions, the effect being greatest in the Limbrick. In general, however, a 10% change in relative humidity at 72° F. had less effect than a 1° F. change in temperature.

TABLE VII

"Experimental error" and net standard error of prediction of vacuum oven percentage moisture by electrical moisture meters

Meter	Moisture range, %	Experimental error (standard error of duplicates), moisture %	Net standard error of prediction, moisture %
	Hard red spring wheat		
Tag-Heppenstall (motor)	11.0 to 17.0 17.0 to 24.0	0.02 0.03	0.16 0.56
Limbrick	11.5 to 17.0 17.0 to 21.5	0.08 0.09	0.38 0.49
Tag-Heppenstall (hand)	11.0 to 17.5 17.0 to 24.0	0.04 0.05	0.40 0.44
Burton-Pitt	11.0 to 17.0	0.06	0.54
	Amber durum wheat		
Tag-Heppenstall (motor)	11.5 to 17.5 17.5 to 22.0	0.02 0.04	0.13 0.10
Limbrick	11.5 to 16.0 16.0 to 19.0	0.04 0.05	0.29 0.30
Burton-Pitt	11.5 to 18.5	0.06	0.44
	Barley		
Tag-Heppenstall (motor)	11.5 to 17.5 17.5 to 25.0	0.04 0.06	0.29 0.34
Limbrick	11.0 to 17.5 17.5 to 20.0	0.07 0.06	0.42 0.50
Burton-Pitt	11.0 to 16.0	0.09	0.83
	Oats		
Limbrick	12.5 to 17.0 17.0 to 22.0	0.12 0.19	0.30 0.57
Burton-Pitt	12.5 to 15.5	0.05	0.39
Tag-Heppenstall (motor)	12.5 to 17.5 17.5 to 22.5	0.07 0.13	0.55 1.00

Standard errors apply to mean of duplicates.

Comparison of the results obtained by the rapid analytical methods and moisture meters shows that the Brown-Duvel is as accurate with hard red spring wheat throughout the entire moisture range tested as the motor-operated Tag-Heppenstall is over the limited range 11.0 to 17.0%. At higher moisture contents, or with any of the other grains examined, the Brown-Duvel method is superior in accuracy to any of the electrical testers. The 130° C. air oven method, using a Wiley mill for grinding, is also more accurate than any of the meters, except the motor-operated Tag-Heppenstall, in the lower moisture range with hard red spring wheat.

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STUDIES ON THE NATURE OF DISEASE RESISTANCE IN CEREALS[1]

I. THE REACTIONS TO RUST OF MATURE AND IMMATURE TISSUES

By Margaret Newton[2] and A. M. Brown[3]

Abstract

A study has been made of the reactions of mature and immature tissues of wheat, oats, barley and rye to different cereal rusts. For a short time before cereal plants come into head, there are parts in the upper half of the culm, *e.g.*, the basal portion of the peduncle and of the flag-leaf sheath, that are growing and elongating very rapidly and are thus composed of soft succulent tissue. Growth in the remainder of the culm is proceeding at a much slower rate and hence in these parts most of the tissue is relatively firm and mature. In varieties and strains of wheat, oats and barley which, when inoculated in the seedling stage in the usual way with *Puccinia graminis*, produce a "1" type of pustule, it has been found that, if the different parts of the culm are inoculated by injection with a suspension of urediospores shortly before the plants come into head, the young, rapidly growing parts are very susceptible while the older, more mature parts are highly resistant. A similar result is obtained with plants possessing mature plant resistance. Both resistant and susceptible tissues are therefore present in the same plant at the same time. The young, rapidly growing parts are also susceptible to a number of rusts which are not natural parasites of the particular cereal: *P. graminis tritici* will attack oats and rye, and *P. graminis avenae* and *P. triticina* will attack barley. As the susceptible parts grow older they become as resistant as the rest of the plant. During their susceptible period they are generally quite effectively protected from infection by plant parts that are resistant.

Introduction

It is now well known that some wheat varieties possess a high degree of resistance to stem rust (*Puccinia graminis tritici* Erikss. and Henn.), but it is not yet clearly understood why such varieties are resistant. The same may be said generally regarding cereals and their rusts. A number of investigators (*6, 7, 12, 13, 14, 18, 19, 20*) have concerned themselves with the problem of the nature of resistance to stem rust, and considerable information has been accumulated regarding the morphology, physiology and chemistry of resistant and susceptible wheat varieties. In general the method followed has been to compare, with respect to morphological, physiological, and chemical aspects, the tissues of resistant and susceptible varieties. As the varieties compared may differ from one another in many other respects than their reaction to rust, certain disadvantages are inherent in such a procedure. If both susceptible and resistant tissues could be found at one and the same time in plants of any particular variety, there would be an obvious advantage in using such a variety for the studies.

In 1930, Goulden, Newton and Brown (11) compared the reactions of 14 wheat varieties at two stages of maturity to 16 forms of *P. graminis tritici* and discovered that in some of these varieties the adult plants possess both susceptible and resistant tissues at the same time. Marquis and Quality

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were found to be resistant in the seedling stage to forms 38 and 53, and were considered resistant also in the adult stage to these forms; but, when plants of these varieties were growing rapidly in the adult stage, pustules of the "4" type developed on them in the region just above the nodes and on that portion of the neck (peduncle) which last emerged from the sheath, although the remaining portions of the plants, similarly inoculated, were quite resistant. As adult plants of Marquis and Quality were found to be susceptible in certain parts and resistant in other parts to these two forms of stem rust the varieties were said to possess "regional resistance".

The finding of susceptible and resistant regions in the same plants of each of these two varieties suggested that a similar condition might be found in some other varieties of wheat, and possibly also in varieties of oats, rye and barley. If susceptible and resistant regions were found to be present in plants of a number of different varieties, these varieties would provide favorable material for investigations by biochemists and plant physiologists on the nature of rust resistance. It seemed desirable therefore to ascertain to what extent this phenomenon is present among varieties of cereals. The present paper presents the results thus far obtained in a study of this phase of the problem, and includes the results reported earlier elsewhere in abstract form (18).

Experimental Methods

As field experiments by the senior author had shown that heavier infections were usually secured when the inoculum was deposited within the tissues than when it was applied in the ordinary manner to the exterior of the plant, in all the experiments here reported the plants were inoculated according to the method suggested by Zehner and Humphrey (27), *i.e.*, by injecting a distilled-water suspension of uredíospores into the plant tissue by means of a hypodermic syringe.

Each internode was inoculated at a point about one inch above the node, the inoculations being made while the plants were still growing vigorously and usually when the heads (enclosed in the sheaths) were only partially developed. During the process of inoculation the syringe was held almost parallel to the plant, the needle entering the tissue at a very sharp angle. At each inoculation the needle passed through two leaf-sheaths, sometimes through three when the inoculation was made above the uppermost node. Thus the inoculum was forced in between the sheaths, and between the sheaths and the culms or heads. Only a small amount of it was lost through leakage from the puncture. Repeated experiments demonstrated that when a small amount of the leaf suspension oozed from the top of the sheaths, the tissue involved was satisfactorily inoculated. It was found more difficult to inoculate the older or lower part of a plant than the younger or upper part. No attempt was made to inject the inoculum into the individual leaves, but the flag leaf, if still enclosed, was automatically inoculated by the suspension which was forced up inside the sheath enveloping this leaf. Immediately after inoculation, the plants were placed on benches in the greenhouses where

they were left until the time arrived for recording the results. All the experiments were carried out in two different sections of the greenhouse, kept at temperatures of 60° F. and 75° F. respectively. About three weeks were required for full rust development in the cooler section, and about two weeks in the warmer section.

The symbols used in recording the types of infection on the different hosts are those originally employed by Stakman and Levine (25) and have the following significance:—

Type 0. Host immune. No uredinia are formed; hypersensitive flecks are frequently present. The reaction is then indicated by ";".

Type 1. Host very resistant. Uredinia are minute, each being surrounded by a well defined necrotic area.

Type 2. Host moderately resistant. Uredinia are small to medium in size, and are commonly found in islands of green tissue surrounded by necrotic halos.

Type 3. Host moderately susceptible. Uredinia are medium in size, and do not coalesce very frequently. Hypersensitiveness is absent, but the pustules may be surrounded by chlorotic areas.

Type 4. Host very susceptible. Uredinia are large and generally confluent. Hypersensitiveness is absent, but slight chlorosis may accompany pustules.

Type x. Host reaction heterogeneous. Uredinia on the same leaf vary in size; frequently all the types of infection are found occurring together on the same leaf.

The signs (=), (−), (±), (+) are used to indicate quantitative variations in the above types.

Regional Susceptibility in Adult Plants of Wheat, Oat and Barley Varieties Possessing Seedling Resistance

Wheat Varieties

For these experiments, wheat varieties and strains were selected which, in the seedling stage, were known to possess resistance to certain physiologic forms. These wheats included (i) those which are immune in the seedling stage, such as Marquis × Kanred to form 21, and Iumillo to form 36; (ii) those which are highly resistant in the seedling stage, such as Khapli to forms 21 and 36, Kota to forms 2 and 38, Marquis to form 38, and Speltz Marz to form 36; (iii) those which are semi-resistant in the seedling stage (*i.e.*, develop an "x" type of infection), such as Marquis to form 120, and Speltz Marz to form 38.

It should, however, be pointed out that the reactions just referred to are those that arise from the application of spores to the exterior of plants, the ordinary routine method of inoculation. Previous experiments with resistant varieties had shown that the two methods of inoculation did not appear to result in the same type of infection.

TABLE I

HOSTS TESTED FOR INDICATIONS OF REGIONAL SUSCEPTIBILITY

Species	Variety	Source
Triticum		
T. compactum	Little Club	R.L.*223, C.I. 4066
T. dicoccum	Khapli	R.L. 563, C.I. 4013
T. durum	Acme	R.L. 566, C.I. 5284
T. durum	Iumillo	R.L. 7
T. durum	Pentad	R.L. 203
T. durum	Speltz Marz	R.L. 569, C.I. 6236
T. vulgare	Chul	R.L. 543
T. vulgare	Garnet	R.L. 15
T. vulgare	Hope	R.L. 209
T. vulgare	H-44-24	R.L. 229
T. vulgare	Kota	R.L. 571, C.I. 5878
T. vulgare	Marquis	R.L. 572, C.I. 3641
T. vulgare	Marquis × Kanred	R.L. 226
Avena		
A. sativa	Anthony	R.L. 1075
A. sativa	Green Russian	R.L. 371
A. sativa	Joanette	R.L. 561
A. sativa	Richland	R.L. 172, C.I. 787
A. sativa	Ruakura	R.L. 345, C.I. 2025
A. sativa	Victory	R.L. 159
A. sativa orientalis	Green Mountain	R.L. 929, C.I. 1892
A. sativa orientalis	White Russian	R.L. 177, C.I. 551
A. sterilis	Belar	C.I. 2760
A. sterilis	Red Rustproof	C.I. 1815
A. sterilis	Sterisel	C.I. 2991
A. sterilis	Sunrise	C.I. 982
Hordeum		
H. vulgare	Manchurian	Minnesota Selection 184
H. vulgare	O.A.C. 21	C.I. 1470
H. vulgare	Peatland	R.L. 656
H. vulgare	Success	C.I. 1808
Secale		
S. cereale	Colorless	Minnesota No. 104
S. cereale	Rosen	Minnesota Accession No. 82
S. cereale	Swedish	Minnesota No. 2

* *R.L. indicates the accession numbers of the plant breeding section of the Dominion Rust Research Laboratory.*

A number of adult plants of other varieties were tested to different forms to which they are wholly susceptible in the seedling stage, but as all parts of such plants became almost equally heavily infected, the varieties were considered useless as indicators of regional susceptibility and were not included in further tests.

From the results presented in Table II, it appears that the method employed in the inoculation of a resistant adult plant modifies to a considerable degree the type of infection that develops on it. When urediospores were applied externally to the whole plant, the germ tubes did not come into contact with the most rapidly growing tissues of the plant and the whole plant ordinarily remained resistant, as Khapli to forms 21 and 36, Kota to forms 2 and 38,

TABLE II

Types of infection produced by physiologic forms of *Puccinia graminis tritici* on leaves and sheaths of wheat varieties, (1) when spores are applied to the surface of mature tissue and (2) when spores are deposited in immediate contact with rapidly growing tissue

Variety	Number of plants tested	Form used	Spores applied to the surface of mature tissue		Spores deposited in immediate contact with rapidly growing tissue			
					Flag leaf		Flag-leaf sheath	
			Leaves	Sheaths	Basal portion	Apical portion	Lower portion	Upper portion
Immune								
Marquis × Kanred	10	21	(0)	(0)	(0) (;)	(0)	(0) (;)	(0)
Iumillo	10	36	(0)	(0)	(0) (;)	(0)	(0) (;)	(0)
Resistant								
Khapli	10	36	(1) −	(1) −	(3) (4)	(;) (1) −	(3) (4)	(;) (1) −
Khapli	16	21	(1) =	(1) =	(3) (4)	(;) (1) −	(3) (4)	(;) (1) −
Kota	10	2	(2) =	(2) =	(3) (4)	(;) (2)	(3) (4)	(;) (2) −
Kota	10	38	(1) +	(1) +	(3) (4)	(;) (1)	(3) (4)	(;) (1)
Marquis	30	38	(2) =	(2) =	(3) (4)	(;) (1)	(3) (4)	(;) (2) −
Speltz Marz	18	36	(1) =	(1) =	(3) (4)	(;) (1)	(4)	(;) (1)
Indeterminate (mesothetic)								
Marquis	16	120	(x)	(x)	(3) (4)	(;) (x) −	(3) (4)	(;) (x) −
Speltz Marz	14	38	(x) +	(x) +	(3) (4)	(;) (2) −	(4)	(;) (1)

Marquis to form 38, and Speltz Marz to form 36. Similarly, when urediospores were applied externally to a semi-resistant plant (one which develops an "x" type of infection), as Marquis to form 120 and Speltz Marz to form 38, the whole plant remained fairly resistant at low or medium temperatures. When, however, the urediospores were injected by means of a hypodermic syringe into the tissues of the different internodes of plants in groups (ii) and (iii) mentioned above, the germ tubes came into contact with very rapidly growing tissue in the upper part of the plants, and some ten days later pustules of the "4" type developed on the sheaths of the upper part of these plants (Fig. 1, A); the sheaths of the lower part, however, were growing very slowly and remained resistant. That is to say, the tissues that were growing and elongating very rapidly were susceptible and large pustules developed upon them, whereas the tissues that were growing very slowly or had ceased to elongate were resistant and remained relatively free from infection.

As already indicated, each node at the time of inoculation by injection was enclosed by a sheath, but, by the time the pustules began to break out on the sheaths of the upper part of the plant, each node had pushed beyond the sheath. The heaviest infections occurred in the flag-leaf sheaths, those on the other sheaths being lighter. If the flag leaf was enclosed at the time of inoculation, a heavy infection developed on its basal portion.

The rust development on any sheath was not uniform with respect to pustule type. From the node upward to the base of the leaf blade there was

FIG. 1, A. *Types of infection on the sheath of Marquis wheat following inoculation by injection with P. graminis tritici form 38, a form to which Marquis is resistant.* FIG. 1, B. *Types of infection on the flag leaf of Peatland barley following inoculation by injection with P. graminis tritici form 21. Note in A and B the gradual transition from the susceptible type of pustule on the younger tissue at the base to the resistant type of pustule on the older tissue above.* FIG. 1, C. *Type of infection on the peduncle of Acme wheat. The peduncle was inoculated with form 21 while it was still very young and enclosed in the sheath. Similar peduncles after they had emerged from the sheath were quite resistant to this form.* FIG. 2, A. *The lower portion of two young culms of Acme wheat inoculated by placing dry spores inside the outer sheath. The white bands are pieces of gummed paper which keep the sheaths in their clasping position.* FIG. 2, B. *Infection on inner sheath above node on Acme wheat resulting from the inoculation mentioned in A. The outer sheath has been cut off a short distance below the node.*

a more or less gradual transition from the susceptible to the resistant type. The susceptible region varied in extent in different plants according to the succulence of the plant. In general, the more rapidly the plant was growing, the larger was the susceptible region; but even in the most rapidly growing plants, the susceptible region did not extend halfway up any internode and, usually, it involved only one or two inches of the sheath. In this region the pustules were of a susceptible or "4" type; while, directly above this region, the pustules became progressively smaller until they were of the resistant or "1" type, such as developed on the seedlings of the varieties in question. The presence of the "4" type of pustule upon the younger tissue of the upper part of the plants, and of the "1" type upon the older tissue seemed to suggest a correlation between the age of the tissue and the ease with which the organism could develop in it.

Although the correlation appeared to hold with all resistant and semi-resistant varieties, it did not seem to hold with the immune strain Marquis × Kanred inoculated with form 21, or Iumillo inoculated with form 36. Such varieties remained resistant despite the age of the tissue inoculated or the method of inoculation. The variety Iumillo was not genetically pure for rust resistance, and, as a consequence, a very small number of plants showing susceptibility were found in some of the trials.

As has already been stated, all the experiments were carried out at both a high (75° F.) and a low temperature (60° F.), for it was known that differences in temperature may bring about striking differences in type of rust infection. Waterhouse (26) working with *P. graminis tritici* called attention to the fact that environmental changes, especially temperature changes, may bring about fluctuations not only in the amount of stem rust development but also in the type of infection produced. Johnson (15) found that any physiologic form of stem rust which produces an "x" type of infection on durum wheats at ordinary greenhouse temperature was likely to produce on the same variety a "4" type of infection at high and a "1" to "0" type at low temperatures. Although, with physiologic forms which produced an "x" type of infection, the response to temperature was more pronounced than with other forms, nevertheless a large number of forms showed at least a slight response to temperature. It seemed quite possible, therefore, that at the two different temperatures (75° F. and 60° F.) the pustule size of the susceptible and resistant portions of the sheaths might not remain constant, especially as two physiologic forms were being used in the tests which produced an "x" type of infection on certain hosts, namely, form 38 on Speltz Marz and form 120 on Marquis. In order, therefore, to obviate any uncertainty in the interpretation of the results of the present experiments the plants were kept at the two temperatures specified.

An examination of the plants maintained at both temperatures disclosed no visible difference in the amount of rust development or in the type of pustule on the susceptible portions of the plants, or any difference in these

respects on the resistant portions. At both temperatures, the susceptible portions were almost covered with pustules of the "4" type, while the resistant portions had scattered pustules of "1" and "x" types.

Oat Varieties

As the experiments had shown that plants of the wheat varieties enumerated in Table II were resistant to certain physiologic forms of *P. graminis tritici* in the seedling stage when spores were applied to their exterior, but susceptible in the adult stage when spores of these same forms were injected into certain regions of the plants, an experiment was begun to discover if resistant varieties of oats might likewise exhibit regional susceptibility when spores of *P. graminis avenae* Erikss. and Henn., were injected into them.

For this experiment the oat varieties Green Russian, Joanette, and Richland were inoculated by injection with *P. graminis avenae* form 1; Belar, Red Rustproof, Ruakura, Sunrise, and Sterilis Selection, with *P. coronata avenae* form 3; and Anthony and White Russian, with *P. coronata avenae* form 4. All these varieties were known to be highly resistant in the seedling stage to the respective forms just enumerated. As will be seen from Table III, heavy infections developed on the culm above the nodes of the upper part of the plant and also on the basal portions of the upper leaves. As in the case of wheat, there was a tendency also for the pustules on the oat varieties to become progressively smaller from the node to the base of the leaf above, and from the base of the leaf to the tip. On an oat plant, however, the susceptible region was somewhat larger than on wheat, a condition probably attributable to the fact that the oat plants were in a more succulent state than were the wheat plants.

The oat experiment was also carried out both at a high (75° F.) and a low temperature (60° F.), in order to ascertain if two different temperatures would bring about any difference in the amount of rust development or in the type of pustule on the susceptible portions of the plants or any difference in these respects on the resistant portions. Peturson (21) had observed striking differences at these two temperatures in infections on seedlings inoculated with forms of *P. coronata avenae* that produce an "x" type of infection, and Gordon (9) had demonstrated a similar behavior for *P. graminis avenae* when forms that produce an "x" type of infection were used. With oats, as with wheat, no discernible differences were observed between the reactions of the susceptible areas, or of the resistant areas, at either temperature.

Barley Variety

As the experiments had shown that both wheat and oat plants in the seedling stage were resistant to certain physiologic forms of stem rust when spores were applied to their exterior, but susceptible when spores of these same forms were injected into them, it seemed quite probable that resistant barley varieties might likewise exhibit a regional susceptibility when spores of *P. graminis tritici* were injected into them.

TABLE III

TYPES OF INFECTION PRODUCED BY PHYSIOLOGIC FORMS OF *Puccinia graminis avenae* AND *P. coronata avenae* ON LEAVES AND SHEATHS OF OAT VARIETIES, (1) WHEN SPORES ARE APPLIED TO THE SURFACE OF MATURE TISSUE AND (2) WHEN SPORES ARE DEPOSITED IN IMMEDIATE CONTACT WITH RAPIDLY GROWING TISSUE

Variety	Number of plants tested	Rust	Form	Spores applied to the surface of mature tissue		Spores deposited in immediate contact with rapidly growing tissue			
						Flag leaf		Flag-leaf sheath	
				Leaves	Sheaths	*Lower portion*	*Upper portion*	*Lower portion*	*Upper portion*
Resistant									
Green Russian	6	*P. graminis avenae*	1	(1) (2) –	(1) (2) –	(3) (4)	(;) (2)	(3) (4)	(;) (2)
Richland	6	*P. graminis avenae*	1	(1) (2)	(1) (2)	(3) (4)	(;) (2)	(3) (4)	(;) (2)
Indeterminate									
Joanette	6	*P. graminis avenae*	1	(x) –	(x) –	(3) (4)	(x)	(3) (4)	(x)
Resistant									
Red Rustproof	4	*P. coronata avenae*	3	(;) (1) –	(;) (1) –	(3) (4)	(;) (1) –	(3) (4)	(;) (1) –
Ruakura	4	*P. coronata avenae*	3	(;) (1)	(;) (1)	(3) (4)	(;) (1)	(3) (4)	(;) (1)
Sunrise	4	*P. coronata avenae*	3	(;) (1) –	(;) (1) –	(3) (4)	(;) (1) –	(3) (4)	(;) (1) –
Sterilis Selection	4	*P. coronata avenae*	3	(;) (1) –	(;) (1) –	(3) (4)	(;) (1) –	(3) (4)	(;) (1) –
Indeterminate (mesothetic)									
Anthony	4	*P. coronata avenae*	4	(x)	(x)	(3) (4)	(x) +	(3) (4)	(x) +
Belar	4	*P. coronata avenae*	3	(x)	(x)	(3) (4)	(x) +	(3) (4)	(x) +
White Russian	4	*P. coronata avenae*	4	(x)	(x)	(3) (4)	(x) +	(3) (4)	(x) +

Only one barley variety, Peatland, was known to be highly resistant to stem rust in the field. It was selected, therefore, for the test. Ten plants were inoculated by the injection method with *P. graminis tritici* form 21 and ten with form 36.

Results similar to those reported for wheat and oats were obtained. The lower part of each plant was resistant but some portions of the upper part were susceptible, especially that portion of the sheath that lies directly above the uppermost node. Numerous pustules of the susceptible type developed on it. There was a tendency also for the pustules to become progressively smaller from the node upwards to the base of the leaf, and from the base of the leaf to the tip (Fig. 1, B).

Regional Susceptibility in Adult Plants of Wheat Varieties Possessing Mature Plant Resistance

Some varieties of wheat in their early stages of growth are susceptible to certain physiologic forms of stem rust, but in later stages become highly resistant. For instance, Melchers and Parker (17) described a strain of Crimean Kansas which gave no evidence of resistance in the greenhouse but was partially resistant in the adult stage in the field. Hursh (14) called attention to the difference between the rust reactions of Acme seedlings and the reactions of that variety when approaching maturity in the field, and attributed these differences to greater amounts of sclerenchyma in the mature plants, which acted as a mechanical barrier to the growth of the rust mycelium. Goulden, Newton and Brown (11) studied the reactions of wheat varieties at two stages of maturity to sixteen physiologic forms of stem rust and, on the basis of their results, were able to divide these varieties into three groups: (1) varieties showing no evidence of mature plant resistance—Garnet, Marquis, Quality, and Khapli; (2) varieties showing varying degrees of mature plant resistance, from a mere indication to a very pronounced evidence—Reward, Kota, Black Persian, Hope, H-44-24, Pentad, and Acme; and (3) varieties showing no appreciable difference between the seedling and mature plant reactions but suspected of possessing considerable mature plant resistance owing to the very low percentage of rust which develops on them in the mature stage—Vernal emmer and Iumillo.

Goulden, Neatby and Welsh (10) in an inheritance study, pointed out that the mature plant resistance in H-44-24 × Marquis crosses and in a Pentad × Marquis cross was inherited independently of the physiological resistance observed in the seedling stage.

As there appeared to be a sharp distinction between the mature plant and the seedling type of resistance, the question naturally arose, would the young, rapidly elongating tissue in adult plants possessing mature plant resistance become infected if spores were deposited in immediate contact with it, as did such tissue in resistant plants possessing seedling resistance?

Adult plants of Hope and H-44-24, varieties known to be highly resistant, and of Pentad and Acme, varieties known to be quite resistant in the mature

stage to stem rust, were inoculated with a suspension of urediospores of form 21 in the manner already described. After the inoculated plants had remained two weeks in the warm house, or three weeks in the cold one, their reaction was very similar to that of the varieties resistant in the seedling stage. The remaining portions of the plants were quite resistant.

The experiment was repeated, but another form, namely, form 36, was used as inoculum. Very similar infection areas to those reported for form 21 developed on all the hosts except Pentad. For some unknown reason Pentad was more lightly infected, and in each test the proportion of plants which became infected was smaller than when form 21 was used.

TABLE IV

TYPES OF INFECTION PRODUCED ON THE LEAVES AND SHEATHS OF FOUR WHEAT VARIETIES POSSESSING MATURE PLANT RESISTANCE WHEN SPORES OF TWO PHYSIOLOGIC FORMS OF *Puccinia graminis tritici* ARE (1) APPLIED TO THE SURFACE OF MATURE TISSUE AND (2) DEPOSITED IN IMMEDIATE CONTACT WITH RAPIDLY GROWING TISSUE

Variety	Number of plants tested	Form used	Spores applied to the surface of mature tissue		Spores deposited in immediate contact with rapidly growing tissue			
					Flag leaf		Flag-leaf sheath	
			Leaves	Sheaths	Lower portion	Upper portion	Lower portion	Upper portion
Acme	30	21	(;) (1) +	(;) (1) +	(4)	(;) (2) +	(4)	(;) (2) +
Hope	40	21	(;) (1)	(;) (1)	(4) –	(;) (2)	(4) –	(;) (2)
H-44-24	40	21	(;) (1)	(;) (1)	(4) –	(;) (2)	(4) –	(;) (2)
Pentad	35	21	(;) (1)	(;) (1)	(4) –	(;) (2)	(4) –	(;) (2)
Acme	30	36	(;) (1) +	(;) (1) +	(4)	(;) (2)	(4)	(;) (2)
Hope	40	36	(;) (1)	(;) (1)	(3) +	(;) (1)	(3) +	(;) (1)
H-44-24	40	36	(;) (1)	(;) (1)	(3) +	(;) (1)	(3) +	(;) (1)
Pentad	60	36	(;) (1)	(;)	(3)	(;) (1)	(3)	(;) (1)

In most cases awns and glumes of plants of the varieties Hope, H-44-24, Acme, and Pentad were also heavily infected by both forms 36 and 21, but the necks were often free from infection. These findings were contrary to our expectations, for, while the necks were still enclosed in the sheaths, they grew extremely rapidly and should have contained a large amount of young tissue. Either the neck tissues were not susceptible or the inoculum did not come into contact with them. In order to determine this point, the sheaths were carefully stripped away from the necks of a number of plants and the necks inoculated in the ordinary way by applying spores to the surface. After the sheaths had been removed, it was found that the necks were too weak to support the heads and required mechanical support. The necks eventually became heavily infected (Fig. 1, C). It seems fairly safe to assume, therefore, that the absence of infection on the necks, already referred to, cannot be attributed to resistance in the neck tissues but rather to the failure of the inoculum to reach those tissues.

In further experiments it was found that, with increasing age, the necks became less and less susceptible and by the time they had fully emerged from the sheath they were highly resistant. The period of extreme susceptibility was thus comparatively short. It varied, however, to some extent with the condition of the plant. When the plant was very succulent and was growing extremely rapidly, the tissues of the neck remained susceptible for a longer period than when the plant was growing more slowly. In other words, regional susceptibility was always associated with young, rapidly growing tissue.

Infections on the Culm above the Node in Field and Greenhouse

Most investigators who have studied the rust reactions of wheat varieties in the field have called attention to the fact that pustules often appear on the culm just above the nodes before any pustules can be found on other parts of the plant.

Hart (13) made a careful morphological study of the sheath above the node as well as of other portions of the sheath and came to the conclusion that the large pustules above the nodes were due to the fact that the sclerenchymatous girders (stereomes) did not extend to the outer epidermis in this part as they did in other parts of the sheath, and, consequently, the mycelium of the rust could spread tangentially as well as lengthwise in the tissue and thus form larger pustules than were possible in the remainder of the sheath.

Her findings might account for the presence of large pustules just above the nodes of susceptible plants, but they did not seem to explain why, in most varieties exposed to natural infection, large pustules often appeared also on the sheaths just below the leaf blade where, as she showed, the stereomes did extend to the outer epidermis; or why, under conditions of natural infection, heavy infections often developed just above the nodes of wheat varieties possessing mature plant resistance, such as Hope and Acme, whose resistance she attributed to the behavior of their stomata.

An explanation was suggested by results already mentioned in this paper: heavy infections were obtained by injecting a suspension of spores inside the leaf sheaths. Might not the large pustules just referred to have arisen from spores which had settled inside the sheath?

Generally, of course, infection is supposed to take place through the stomata of the outer epidermis of the plants; and, as a rule, the sheaths, especially the upper ones, tightly enfold the culm so that the possibility of spores getting inside a sheath may appear somewhat remote. However, under field conditions, winds sway the plants to and fro, causing them to bend. Sometimes the leaves of one plant get entangled with the leaves and culms of other plants. There would, therefore, be a tendency for the leaf sheaths to become loosened somewhat from the culms, or drawn away from them, at least for periods of short duration. In such an eventuality the spores might fall, or be washed down inside the sheaths by dew or rain drops.

It has already been mentioned in this paper that until a wheat plant is about to come into head the leaf sheath originating at a node (except the uppermost one) extends upwards about one inch or more beyond the node next above, so that the latter node and the lower portion of the sheath arising from it are enfolded for a considerable length of time by the upper portion of the first-mentioned sheath. If, therefore, spores succeeded in getting inside the upper part of this sheath, they would have an opportunity, after germination, to infect the upper portion of this sheath and the basal portion (enfolded by it) of the other one. Subsequently, as the plant grew, the two infected areas would be gradually drawn apart, so that the infections on the two areas would appear to have originated from two entirely different lots of spores.

In order to ascertain if heavy infections would develop on the upper portion of one sheath and on the basal portion of the sheath next above if dry urediospores were deposited inside the former, the sheaths of plants of Hope, H-44-24, Acme and Pentad were carefully drawn away sufficiently far from the culms to permit the depositing of urediospores inside them. Some plants of each variety were inoculated in this manner with form 21 and others with form 36. The sheaths were then replaced in their normal clasping position. The ones on the upper parts of the plants held that position without difficulty, but the ones on the lower parts of the plants had to be kept in place by mucilaged bands (Fig. 2, A). Owing to transpiration there was always some moisture inside the sheaths so that once the spores had been deposited they germinated readily.

As has been stated, the nodes were still enclosed in the sheaths at the time of the inoculations, but in less than ten days they emerged from the sheaths, for at this time very rapid growth is taking place in the nodal regions, particularly of the upper part of the plant. By the time the nodes had emerged, heavy infections (Fig. 2, B) were found to be developing just above the nodes of the upper part of the plant, the heaviest infections being just above the topmost node. These infections were similar to those which had appeared on the same wheat varieties when a distilled-water suspension of urediospores was injected into the tissues. In many cases a heavy infection also occurred on the upper part of the sheaths, the portion inside of which the spores had been deposited. Apparently in these cases infection had taken place on the inner side of the sheaths and the rust mycelium had spread through the sheath tissue to form pustules on the outer surface. It, therefore, seems safe to assume that many of the large pustules which, under field conditions, develop just above the nodes and on the upper portion of the leaf sheaths arise from infections produced by spores which succeed in getting inside the leaf sheaths.

Infection of Cereals by Rusts that are not Natural Parasites on them

It has long been known that the germ tubes of spores of different rusts can enter the stomata of plants that are not natural hosts for such rusts and reach

a certain stage of development in the substomatal cavity, but, as far as we are aware, it has not yet been reported that such incipient infections ever develop further.

In 1905, Gibson (8) inoculated a large number of unrelated plants on their outer surfaces with spores of *Puccinia chrysanthemi* Roze, as well as of other rusts, and found that the germ tubes enter the stomata of the different plants in the same way as they do in the natural hosts of these rusts. Substomatal vesicles are formed which give rise to from one to six hyphae. However, in the subsequent course of development the hyphae do not form haustoria and in about four days lose their capacity for growth. She concluded that whenever a germ tube of any rust fungus enters any plant other than a natural host a struggle goes on resulting in the death both of the host (locally) and of the parasite.

Stakman (23) and Stakman and Piemeisel (24) inoculated certain cereals and grasses on the outer surfaces with *Puccinia graminis* and found that some of these hosts could be attacked by certain rusts that are not, normally, parasitic on them. For example, rye and oats were attacked very slightly by *P. graminis tritici*, and rye and barley, slightly by *P. graminis avenae*, very minute pustules being formed in each case.

As the infections by *P. graminis avenae* on oats and rye reported by Stakman, and Stakman and Piemeisel were really quite similar to the type of infection that occurs on any cereal when inoculated on the surface with a physiologic form to which it is resistant, it seemed to us quite possible that heavy infections would develop on oat and rye plants, as on resistant wheat plants, if spores of *P. graminis tritici* were deposited in close contact with the rapidly growing tissues of such plants; and, similarly, that heavy infections would develop on rye and barley if thus inoculated with spores of *P. graminis avenae*.

A large number of such tests were carried out using the above-named hosts and rusts, as well as some additional hosts and rusts. The results of some of these tests are presented in Table V. From them it will be seen, for example, that the young tissues of the adult plants of oats and rye were heavily attacked by *P. graminis tritici* forms 9, 21 and 36; and barley by *P. graminis avenae* forms 6, 7 and 8, and *P. triticina* forms 20 and 35, but the tissues of wheat remained resistant to *P. graminis avenae* forms 6, 7 and 8, and those of barley resistant to *P. coronata avenae* forms 3 and 4. As far as the work has gone the results seem to show that if a plant in the seedling stage is immune when inoculated on the surface with a certain rust, infection being indicated only by flecking, injections of this rust into the young tissues of the plant will not result in a susceptible type of infection; but, if, on the other hand, minute pustules develop on a plant in the seedling stage after being inoculated on the surface with a certain rust, a "4" type of pustule will develop above the upper nodes of such a plant if the inoculum is deposited in close contact with the young tissues.

TABLE V

TYPES OF INFECTION PRODUCED BY CERTAIN RUSTS ON CEREALS THAT ARE NOT THEIR NATURAL HOSTS

Variety	Rust used	Forms	Spores applied to the surface of mature tissue		Spores deposited in immediate contact with rapidly growing tissue			
					Flag leaf		Flag-leaf sheath	
			Leaves	Sheaths	Lower portion	Upper portion	Lower portion	Upper portion
Wheat								
Little Club	*P. gr. avenae*	6, 7, 8	(;)	(;)	(;) (1)	(;)	(;) (;)	(;)
Marquis × Kanred	*P. gr. avenae*	6, 7, 8	(;)	(;)	(;) (1)	(;)	(;) (1)	(;)
Speltz Marz	*P. gr. avenae*	6, 7, 8	(;)	(;)	(;) (1)	(;)	(;) (1)	(;)
Chul	*P. anomala*	1, 2	(;)	(;)	(;)	(;)	(;)	(;)
Garnet	*P. anomala*	1, 2	(;)	(;)	(;)	(;)	(;)	(;)
Little Club	*P. anomala*	1, 2	(;)	(;)	(;)	(;)	(;)	(;)
Oats								
Green Mountain	*P. gr. tritici*	9, 21, 36	(;) (1)	(;) (1)	(3) (4)	(;) (1)	(3) (4)	(;) (1)
Ruakura	*P. gr. tritici*	9, 21, 36	(;) (1)	(;) (1)	(3) (4)	(;) (1)	(3) (4)	(;) (1)
Victory	*P. gr. tritici*	9, 21, 36	(;) (1)	(;) (1)	(3) (4)	(;) (1)	(3) (4)	(;) (1)
Ruakura	*P. triticina*	20, 35	(;)	(;)	(;)	(;)	(;)	(;)
Victory	*P. triticina*	20, 35	(;)	(;)	(;) (1)	(;)	(;) (1)	(;)
Barley								
Manchuria	*P. gr. avenae*	6, 7, 8	(;) (1)	(;) (1)	(3) (4)	(;) (1)	(3) (4)	(;) (1)
O.A.C. 21	*P. gr. avenae*	6, 7, 8	(;) (1)	(;) (1)	(3) (4)	(;) (1)	(3) (4)	(;) (1)
Success	*P. gr. avenae*	6, 7, 8	(;) (1)	(;) (1)	(3) (4)	(;) (1)	(3) (4)	(;) (1)
Manchuria	*P. triticina*	6, 7, 8	(;) (1)	(;) (1)	(3) (4)	(;) (1)	(3) (4)	(;) (1)
O.A.C. 21	*P. triticina*	20, 35	(;) (1)	(;) (1)	(3) (4)	(;) (1)	(3) (4)	(;) (1)
Success	*P. triticina*	20, 35	(;) (1)	(;) (1)	(3) (4)	(;) (1)	(3) (4)	(;) (1)
Manchuria	*P. coronata avenae*	3, 4	(;)	(;)	(;) (1)	(;)	(;) (1)	(;)
O.A.C. 21	*P. coronata avenae*	3, 4	(;)	(;)	(;) (1)	(;)	(;) (1)	(;)
O.A.C. 21	*P. helianthi*		(;)	(;)	(;)	(;)	(;)	(;)
O.A.C. 21	*P. taraxaci*		(;)	(;)	(;)	(;)	(;)	(;)
Rye								
Rosen	*P. gr. tritici*	9, 21, 36	(;)	(;)	(2) (3)	(;)	(2) (3)	(;)
Swedish	*P. gr. tritici*	9, 21, 36	(;)	(;)	(2) (3)	(;)	(2) (3)	(;)
Colorless	*P. gr. tritici*	9, 21, 36	(;)	(;)	(2) (3)	(;)	(2) (3)	(;)

Discussion

Many varieties of wheat are known to be resistant to one or more physiologic forms of stem rust and to be susceptible to other forms. A few varieties are susceptible in the seedling stage to certain forms but highly resistant in the adult stage to all forms. On the other hand, it has been affirmed (5) that the susceptibility of a variety increases with increasing age of the plants. In the present investigation, it is not so much a matter of varietal resistance or susceptibility to particular forms of stem rust, or of the resistance or susceptibility of plants at different stages of development, but of some portions of the same plant being highly resistant and other portions highly susceptible at one and the same time.

The fact that the young, rapidly growing portions of rust-resistant varieties and strains of cereals are susceptible to stem rust has little or no significance

with respect to the ability of these varieties and strains to withstand the disease under field conditions. The susceptible portions of the plants are generally quite effectively protected by plant parts that are highly resistant. Although, as already mentioned, large pustules of stem rust may occasionally occur above some nodes of the resistant plants, the yield of the plants thus affected is not reduced in any measurable degree.

Whether or not the young tissues of an adult plant are susceptible to a physiologic form or even to a different rust can usually be ascertained in advance by observing the infection type on the seedling plant following inoculation with the particular physiologic form or rust. In general it was found that if a plant in the seedling stage is immune when inoculated on the surface with a certain physiologic form or rust, infection being indicated only by flecking, injection of this rust into the young tissues of the plant will not result in a susceptible type of infection; but, if, on the other hand, minute pustules develop on a plant in the seedling stage after being inoculated on the surface with a certain rust, a "4" type of pustule will develop on the very rapidly growing portion of such a plant if the inoculum is deposited in close contact with the young tissue.

That young tissues should be more susceptible than older tissues to attacks by rust is not surprising for it has been repeatedly shown, with plants other than cereals, that immature leaves and tissues are often more susceptible to certain diseases than older tissues. For instance, Salmon (22) found that the fully matured leaves of *Euonymus japonicus* L. did not become infected by mildew (an obligate parasite like the rusts) although the young leaves were quite susceptible. Ballard and Volck (1) observed that the young leaves and twigs of apple trees are particularly heavily infected with mildew. It is known also that young leaves of *Berberis vulgaris* L. are susceptible to *P. graminis* while the older ones are resistant. In these instances, however, the cuticle of the leaf is penetrated directly by the organism while in cereals the rust organism enters by way of the stomatal openings. The cases cited, therefore, may be in no way comparable with the present one. A mechanical mode of penetration suggests, of course, a mechanical basis of resistance, although this does not necessarily follow.

A somewhat parallel case to that of young susceptible tissues in cereals is reported by Fitzpatrick (4). He found that *Taphrina deformans* (Fcl.) Tul. will not spread in the peach leaf after the latter has reached a certain stage of maturity and he concluded that the older tissues develop a definite resistance to the organism.

The nature of resistance to stem rust is very imperfectly or not at all understood. The resistance of some wheat varieties has been attributed, in part at least, (*a*) to the late opening of the stomata (13); (*b*) to the high ratio of sclerenchymatous to collenchymatous tissue in the culms (14); (*c*) to the production of some toxic substance by the host tissue in the immediate vicinity of the infections (3, 19, 20); and (*d*) to a lack of suitable nutriment in the host tissue for the fungus (7, 16). It is not proposed to review here

the work of the various investigators who have concerned themselves with the problem of the nature of resistance to stem rust. The literature has been recently reviewed by Chester (2). Mention will only be made of two investigations which may appear to have some bearing on the present work.

Hart (13) found that the stomata of some varieties that possess mature plant resistance, Hope and Acme, open later in the morning than those of the susceptible varieties, Little Club and Marquis, and she formulated the theory that the resistance of Hope and Acme in the field is due principally to the behavior of their stomata. As has already been stated, when plants of Hope and Acme were inoculated by injection before they came into head, pustules developed upon the sheaths of the upper portion of the plants, particularly upon the flag-leaf sheath. It will be remembered that at the time of inoculation, when the inoculum was being forced between the sheaths, the flag-leaf sheath was covered by an outer sheath but, by the time the pustules appeared, the flag-leaf sheath had pushed beyond this outer sheath into full view. The pustules on the flag-leaf sheath were not all of uniform size. Upon the susceptible (young) tissue just above the node the pustules were of a "4" type, while, upon the resistant (older) tissue higher up on the same sheath, they were of a "1" type. As pustules occurred both on the susceptible and resistant portion of the sheath, it would seem that resistance in the sheath of Hope and Acme cannot be due to the failure of the germ tubes to enter the stomata of the sheath but rather to some characteristic of the cells or of the protoplasm.

It has been shown by Hursh (14) that the internal structure of the plant may affect the relative shape of the pustules. He found that, in the peduncle of Kota wheat, broad strands of sclerenchyma separate the narrow strands of collenchyma and that when the peduncles were attacked by rust, the pustules, hemmed in on two sides by sclerenchymatous strands and unable to grow at right angles to the peduncle, developed into long narrow pustules rather than large and broad pustules characteristic of susceptible wheats. In the resistant portion of the sheath and peduncle of the plants inoculated by injection there was nothing in the shape of the pustule to suggest mechanical interference. The pustules were of the normal "1" type which occurs on resistant seedlings and were so minute that the morphology of the leaf or peduncle would not appear to have influenced their shape.

Hursh (14) has also shown that there is a high ratio of sclerenchymatous to collenchymatous tissue in the culm of some resistant wheats. In the present investigation a study was not made of the morphology of the susceptible tissues in the resistant plants tested, but it was found that prior to the emergence of the heads, the peduncles of the plants were very flexible and quite unable to support the heads without mechanical support. At this stage the peduncles were very susceptible. As they became older, and more rigid, they became highly resistant. Similarly the young, rapidly elongating, basal portion of the sheath was always susceptible while the older portion was resistant.

Susceptibility in these young plant parts where sclerenchymatous tissue is apparently not well developed and resistance in the older parts where this tissue must be well developed would seem to indicate that mechanical resistance was operative. However, some of the results recorded in this paper throw considerable doubt on this conclusion. The young tissues of all wheat varieties are not susceptible to all physiologic forms of stem rust, although susceptible to many forms. For instance, the young tissues of the immune strain Marquis × Kanred remain resistant to form 21 despite the age of the tissue inoculated or the method of inoculation used. Furthermore, the young tissues of a cereal, such as oats or barley, may be susceptible to a rust which is not a natural parasite on it, but the same tissue may be highly resistant to another rust which is also not a natural parasite of that cereal. The young tissues of the oat variety Ruakura are heavily attacked by *P. graminis tritici* forms 9, 21 and 36 but they are highly resistant to *P. triticina* forms 20 and 35. If, in these two instances, the resistance is due to mechanical obstruction, it is difficult to explain why the young tissues should retain their resistance only to certain physiologic forms and rusts and not to others, for such obstructions should be effective against any form or rust.

The present experiments have shown that the young tissues of resistant wheat and barley plants are (with the exceptions mentioned earlier) susceptible to physiologic forms of wheat stem rust, and young tissues of resistant oat plants to stem rust of wheat as well as of oats. Furthermore, the young tissues of barley plants are susceptible to stem rust of oats and leaf rust of wheat. When the young tissues grow older and are therefore more fully differentiated, they become highly resistant to the physiologic forms or rusts indicated. As already implied, this resistance does not appear to be morphologic in nature. It seems more probable that, with advancing age, the protoplasm of the cells undergoes some change which renders the tissues highly resistant.

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STUDIES ON THE NATURE OF DISEASE RESISTANCE IN CEREALS

II. THE RELATIONSHIP BETWEEN SUGAR CONTENT AND REACTION TO STEM RUST OF MATURE AND IMMATURE TISSUES OF THE WHEAT PLANT[1]

By T. Johnson[2] and O. Johnson[3]

Abstract

In Part I of these studies it has been shown that the rapidly growing tissues of the wheat plant are more susceptible to stem rust than the older tissues. An attempt was made to discover if a physiological or chemical basis could be found for the difference in reaction of the young and older tissues.

Analyses were made to determine the sugar content of young (susceptible) and older (resistant) tissues of four wheat varieties resistant in the adult stage and of the corresponding plant parts of three wheat varieties which in the adult stage showed little or no resistance to rust. The young tissues comprised the young leaves still enfolded by the uppermost sheaths and the young stems below the uppermost node; the older tissues were represented by the fully developed upper leaves and their adherent sheaths. The analyses showed a considerably higher content of sugars in the young than in the older tissues of the seven varieties tested. The difference was particularly great in the content of reducing sugars but rather slight in the disaccharide content (expressed as invert sugar). However, as all the varieties, irrespective of resistance or susceptibility to rust in the adult stage, showed much the same difference in the sugar content of their young and older tissues, it does not seem likely that there is any direct relation between sugar content and reaction to rust.

Introduction

It has been demonstrated, in Part I of these studies (3), that the rapidly growing tissues of the wheat plant are decidedly more susceptible to stem rust than the older tissues. The work presented here originated as an attempt to discover if a physiological or chemical basis could be found for this radical difference in reaction.

The possibility that differences might exist between the concentration of sugar in the older, but photosynthetically active, parts of the plant and the younger, rapidly growing parts was first suggested by some experiments on the comparative osmotic values of these tissues. Sap was expressed, at a pressure of 6,000 lb. per sq. in., from fully developed leaves, and from young leaves still folded within the uppermost sheaths of wheat plants grown in the greenhouse. The osmotic pressure (determined by the vapor pressure method of Barger (1)) of the sap expressed from the young leaves proved to be considerably greater than that of the sap expressed from the fully mature leaves. The osmotic pressure of the sap expressed from fully developed leaves of the highly resistant varieties Acme, Iumillo, and H-44-24, was found equivalent to 6.7 atm. while the corresponding figure for the sap of the young leaves was 11.2 atm. The susceptible variety Little Club showed somewhat

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similar, but slightly lower, osmotic values. The difference in the osmotic values of the two tissues, about 4.5 atm., was sufficiently great to indicate the possibility of some considerable difference in sugar concentration.

That carbohydrates play an important role in the development of rust is generally recognized. Waters (6), as a result of experiments on the uredio-spore and teliospore formation of ten species of rust, concluded that "all the rusts studied are directly dependent upon the photosynthetic activity of the host". Mains (2), however, has shown that *Puccinia Sorghi* Schw. and *Puccinia coronata* Cda. can develop in the absence of light when sugars are added to the nutrient solutions supplied to the host plants. It was, therefore, thought possible that a correlation might be found between the amounts of sugar present in the different parts of wheat plants and the reaction to stem rust of these plant parts. If, for example, a variety, such as Hope, were found to contain far greater quantities of sugar in its susceptible than in its resistant parts, while a variety, such as Little Club, which is more homogeneous in its reaction to rust, were found to be rather homogeneous in its sugar content also, one might be justified in drawing the conclusion that there was a positive correlation between the reaction of the various plant tissues to rust and their sugar content.

With a view of determining whether or not such a correlation existed, several wheat varieties which were thought to be suitable were selected and grown in plots adjacent to the laboratory to provide material for analyses of the sugar content of the various tissues. The selected varieties included four varieties possessing what is commonly described as mature plant resistance, namely, two varieties of *Triticum vulgare*, Hope and H-44-24, and two varieties of *Triticum durum*, Acme and Pentad. These wheats, as they approach the heading stage, are highly resistant to stem rust in their exposed plant parts but show susceptibility in the partly differentiated and young tissues protected by the outer sheaths. There were also included two varieties of *Triticum vulgare*, Marquis and Garnet, which do not possess mature plant resistance and are therefore susceptible to a great number, although not to all, of the physiologic forms of wheat stem rust. Finally, there was also included the variety Little Club (*Triticum compactum*) which is susceptible in all its stages of development and in all regions of the plant to the great majority of the known physiologic forms of *Puccinia graminis tritici*.

The homogeneity of the reaction of the various plant parts of Little Club, Marquis, and Garnet, holds, however, only for the physiologic forms to which they are susceptible. Experiments with forms to which their plant parts, when inoculated on the surface, are resistant, show that the rapidly growing regions protected by sheaths are only moderately resistant or even susceptible when inoculated by injection (3).

Methods

Plant material was collected for analysis in the last week of June, 1933, all collections being made at the same time of day, between 1:30 and 2:00 p.m.

Each of the seven varieties used was collected at approximately the same stage of development, that is, prior to the appearance of the flag leaf. At the time of collection the young head—in most cases less than one inch in length—was still enfolded within the lower part of the then uppermost sheath of the plant. There was therefore a considerable amount of rapidly growing tissue within this sheath and the one next below it, namely, the leaves folded within the uppermost sheath and the tender, young stem below the uppermost node.

The problem of separating the young, rapidly growing tissues from the fully developed tissues presented greater difficulties and greater opportunities for error than any other phase of the work. There is clearly no point of sharp demarcation between young and fully developed tissue and hence it is evident that any separation of such tissues must be more or less arbitrary. In the procedure adopted, the whole plant was not used for analysis, only such plant parts being used as were definitely known to be, respectively, mature and immature. As mature plant parts, the fully developed upper leaves of the plant together with their adherent sheaths were selected for analysis, while the immature plant parts comprised the young leaves still enfolded by the uppermost sheath and the young stem below the uppermost node.

The procedure actually used was briefly as follows. The plants were cut off near the base, placed upright in a large glass jar containing a quarter of an inch of water, and brought into a cool shady room where the tissues were separated out as quickly as possible. The leaves with their adherent sheaths were stripped off and dropped into a covered glass jar lined with moist blotting paper which maintained a humidity high enough to prevent a serious loss of water from the detached leaves. The young tissues, folded leaves and young stems, were separated out as rapidly as possible and dropped into a similarly prepared glass jar. When about 45 gm. of each of the two tissues had been collected (an operation requiring about 20 min. for two men), both lots were cut into short lengths with scissors, and 25 gm. of each was weighed out for analysis while three 5-gm. portions were used for dry-weight determinations. The 25-gm. lots to be used for analysis were immediately dropped into Erlenmeyer flasks containing 1 gm. of powdered calcium carbonate and 105 cc. of 95% ethyl alcohol. A reflux condenser was attached to the flask and the contents was boiled gently for 30 min. over a steam bath. Samples which could not immediately be subjected to extraction and analysis were stored in a refrigerator at about 5° C. until extraction could be carried out.

Extraction of sugars was performed in a continuous-extraction apparatus of the Soxhlet type, as modified by Newton (4). The sugars were extracted with 85% ethyl alcohol for a period of about 30 hr. The extract was transferred to a Claissen distilling flask, and concentrated under diminished pressure over a steam bath. About 200 cc. of distilled water was added when the contents of the distilling flask had been reduced to a volume of about 75 cc., and further concentration was carried out. The re-concentrated extract was then transferred to a 250 cc. volumetric flask, the concentrate being filtered through a pad of cheesecloth. Clarification was carried out by

adding 5 cc. of a saturated solution of neutral lead acetate, a quantity sufficient to leave in the solution a slight excess of lead acetate. The solution was then made up to volume with distilled water at 20° C. and filtered through a dry filter to remove the precipitate. The filtrate was deleaded by the use of a minimum quantity of powdered sodium oxalate which was added in small portions until precipitation was complete. The solution was then filtered through a dry filter and the filtrate used for the determination of reducing and total sugars. For the determination of total sugars, inversion was carried out by adding 5 cc. of concentrated hydrochloric acid to a 50 cc. aliquot of the neutralized filtrate which was then allowed to stand at room temperature (about 21–24° C.) for 24 hr. A single inversion was made for each extract and the determinations of reducing sugars before and after inversion were carried out in duplicate according to the Quisumbing and Thomas (5) procedure, employing direct weighing of the cuprous oxide.

TABLE I

SUGAR PRESENT IN YOUNG AND OLDER TISSUES OF WHEAT VARIETIES

—	Variety	Type of tissue	Green weight				Dry weight	
			Reducing sugars as dextrose, %	Ratio Young/Old	Sucrose as invert sugar, %	Ratio Young/Old	Reducing sugars as dextrose, %	Sucrose as invert sugar, %
Varieties developing mature plant resistance	Hope	Young	1.11	4.44	0.42	0.84	7.25	2.74
		Old	0.25		0.50		1.21	2.43
	H-44-24	Young	2.58	6.79	0.87	1.61	14.12	4.76
		Old	0.38		0.54		1.75	2.46
	Acme	Young	1.39	3.02	0.81	0.82	8.55	4.97
		Old	0.46		0.99		2.19	4.72
	Pentad	Young	2.25	2.74	1.24	1.17	12.29	6.77
		Old	0.82		1.06		3.95	5.10
Varieties not developing mature plant resistance	Marquis	Young	2.62	5.69	1.24	1.70	14.99	7.09
		Old	0.46		0.73		2.08	3.16
	Garnet	Young	2.40	4.61	1.19	1.65	13.86	6.87
		Old	0.52		0.72		2.27	3.11
	Little Club	Young	1.58	3.51	0.75	1.03	9.97	4.71
		Old	0.45		0.73		2.30	3.72

Discussion of Results

Before entering into a discussion of the possibility of a relationship between the sugar content of plant parts and their reaction to rust, attention should be called to the rather striking differences between the sugar contents of the varieties tested (Table I). The reason for these differences is not clear

although there are at least three possible causal factors. The differences may be due (i) to varietal characteristics, (ii) to the fact that the different varieties were collected for analysis on different days and under slightly different weather conditions, (iii) to slight differences in the age of the material collected. If the differences in sugar concentration were varietal characteristics, it would be expected that two varieties as closely related and similar in appearance as Hope and H-44-24 would also show a somewhat similar sugar content, and that the *vulgare* varieties Marquis and Garnet or the *durum* varieties Acme and Pentad would show some similarities in this respect. The data in Table I do not indicate any close resemblance in the sugar content of related varieties with the exception of the *vulgare* varieties Marquis and Garnet which give very similar results. There is therefore not much evidence that the differences are varietal characters. The second possibility, namely, that the environmental conditions at the time of collection of the plant material modified its sugar content to some extent cannot be overlooked. Although the plant material was in every case collected at the same time of day it is possible that the weather conditions from day to day varied sufficiently to affect to some degree the sugar content of the plants. Finally it is possible that slight differences in age of the plant parts at the time of collection had some influence on their sugar content. This would apply particularly to the younger or immature plant parts which, as already stated, had to be selected more or less arbitrarily. The varietal differences in sugar content are not, however, of any great significance in the present study, the object of which was not a comparison of the sugar content of the different varieties but rather a comparison of the sugar content of two different types of tissue in the same plant.

It is evident from an examination of Table I that the concentration of sugars is decidedly greater in the immature tissues than in the more mature tissues of the wheat varieties investigated. The differences in the amounts of reducing sugars present in the two types of tissues are particularly striking, these sugars being especially abundant, as might be expected, in the less mature regions of the plant, where rapid growth is taking place. To facilitate a comparison, the relationship between the sugar content of the young and older tissues of each variety has been expressed in the form of ratios. Thus in the variety Hope there is 4.44 times as much reducing sugar present in the young as in the older tissue. In view of these differences it would appear that there is some relation between the reaction to rust and the sugar content of the tissues in question, as the plant parts high in sugar content are also susceptible to rust. But all the varieties studied, irrespective of whether they do or do not possess mature plant resistance, show much the same differences in the sugar content of the two types of tissues. Further, it will be observed that, in the varieties Marquis, Garnet, and Little Club, the older plant parts, which are also susceptible to rust, are comparatively low in sugar content; in fact, their sugar content is no higher than that of the corresponding, but highly resistant, plant parts of Acme and Pentad. These observa-

tions throw considerable doubt on the existence of any relation between susceptibility to rust and sugar content, a doubt further confirmed by a consideration of the ratios in column 5 of Table I.

It is obvious from an examination of these ratios that the varieties do not fall into groups according to their rust reaction. In the comparison of the reducing-sugar contents of the two types of tissues, the highest ratio, 6.79, is found in the highly resistant variety H-44-24 which is, however, followed by the susceptible varieties, Marquis with a ratio of 5.69, and Garnet with 4.61. The lowest ratios are given by the resistant varieties Pentad, 2.74, and Acme, 3.02. Similarly, an examination of the ratios for the comparative contents of sucrose fails to show any grouping of these varieties according to reaction to rust. It does not appear, therefore, from this point of view, that there is any evidence that the differences in the sugar content of young and older regions of the plant have any direct relation to the reaction of these regions to stem rust.

Although the above conclusion seems valid for most of the physiologic forms of the stem-rust organism, it does not seem to hold for all the known physiologic forms. Certain forms, to which the leaves of Marquis, Garnet and Little Club are resistant, are capable of attacking, to a certain extent at least, the younger plant parts enclosed within the sheaths. As these younger regions are higher in sugar content than the leaves it follows that, in these instances, there is a correlation between sugar content and reaction to rust. It should be pointed out, however, that there is no proof that the higher sugar content of the younger plant parts is the cause of their greater susceptibility.

Apart from any consideration of its relationship to rust reaction, the preponderance of sugars in the rapidly growing tissues of the wheat plant is of some interest. It accords with the expectations based on the osmotic pressure determinations previously mentioned and is one of the factors contributing to the higher osmotic values of the sap extracted from the younger tissues. As the osmotic values of the extract were considerably lower for the older tissues (photosynthetically active leaves) than for the younger tissues, it follows that if dissolved materials are moving from the leaves to the rapidly growing plant parts they must be moving against an osmotic gradient. The results reported in the present paper indicate not only translocation of sugars against an osmotic gradient but also against a concentration gradient of sugars.

In addition to the differences in total sugar content of the young and older plant parts there is also a considerable difference in the quantitative relationships of disaccharides and monosaccharides in the two types of tissue. In the older tissues the proportion of sugars present as disaccharides is without exception greater than in the younger, namely, from 25.22 to 36.82% in the young tissues, and from 56.38 to 68.27% in the older tissues of the seven varieties tested.

Acknowledgments

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A SIMPLE METHOD FOR DETERMINING THE RELATIVE WEIGHT PER BUSHEL OF THE GRAIN FROM INDIVIDUAL WHEAT PLANTS[1]

By O. S. Aamodt[2] and J. H. Torrie[3]

Abstract

The kernels from individual plants in studies on breeding for drought resistance varied so greatly in size that the usual measure of plumpness (weight per thousand kernels) was of little value. A method for determining the weight per measured bushel of small samples was developed and found to have a very high degree of association with an estimated degree of kernel plumpness. The weight in grams of a 4 cc. sample multiplied by 20, gave a close approximation to the test weight per bushel as determined by the usual apparatus. The correlation coefficient calculated from the weight per bushel of 184 samples of spring wheat by these two methods was $+0.947 \pm 0.005$ and for 59 samples of winter wheat $+0.834 \pm 0.027$.

In the course of drought breeding studies carried on at the University of Alberta, it was found advisable to devise some simple method for determining the relative weight per bushel of the grain from individual wheat plants. The weight per bushel gives an excellent measure of the plumpness of grain for any given sample of wheat. In studying plumpness with large samples several indicators are commonly used, namely, test weight per bushel, weight per thousand kernels and the percentage of plump kernels.

Weight per bushel as an index of flour yielding capacity of wheat was used by the practical miller before the advent of the present grading system. It is now regarded as an important factor in determining the yield of flour to be expected from a sample of wheat. The grading systems in use in both Canada and the United States give the test weight per bushel an important place in determining the grade of the wheat. Kernel plumpness, a desirable feature of wheat from the miller's point of view, is difficult to measure accurately, but, as shown later in this paper, is correlated with weight per bushel. Weight per thousand kernels is not, as far as the writers know, closely related to the flour yielding capacity or general milling quality of a wheat.

Bridgford and Hayes (1) with rod-row trials of spring wheat found a correlation of $+0.36 \pm 0.08$ between per cent plumpness and weight per thousand kernels. Clark and Smith (2) studying the F_3 of crosses between Pentad and Akrona report the following correlations: between test weight per bushel and plumpness percentage, $+0.627 \pm 0.048$; between test weight per bushel and weight per thousand kernels, $+0.468 \pm 0.062$; and between weight per thousand kernels and plumpness percentage, $+0.635 \pm 0.034$. Waldron (3) with rod-row trials, under conditions of high temperature and low moisture,

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obtained the following correlations: between test weight per bushel and plumpness percentage, +0.664; between test weight per bushel and weight per thousand kernels, +0.341; and between weight per thousand kernels and plumpness percentage, +0.630.

The parents used in the drought studies differed considerably in shape and size of the kernel. Milturum .0321 has a long, narrow kernel, while that of Selection I-28-60 is relatively short and broad. The hybrids showed considerable variation in kernel shape and size. Some samples with large, badly shrivelled kernels had the same weight per thousand kernels as other samples with small plump kernels. Weight per thousand kernels does not differentiate between the two characters of kernel plumpness and size. It is also a laborious method involving considerable time and expense. For these reasons the weight per thousand kernels was not considered to be a satisfactory measure of kernel plumpness.

Thus the necessity arose of devising some simple method which would measure these two characters for a sample of grain from a single plant, and at the same time overcome the discrepancy introduced into the determination of plumpness by differences in kernel size. Since, commercially, wheats are evaluated to a considerable extent by their weight per measured bushel, it seemed desirable to determine the weight of these small samples in units that could readily be converted by a simple conversion factor into weight in pounds per measured bushel.

Many of the grain samples from individual plants grown in the drought area had a volume of only a few cubic centimetres. Consequently, the maximum size of the measure to be used for obtaining the relative weight of the grain would be determined by the amount of grain produced by the plants most severely affected by the drought. Those plants that had the kernels developed sufficiently well to be considered as having produced a crop of grain, would have a minimum volume of approximately 4–5 cc. Since there are 36,369 cc. in a bushel, and 453.6 grams in a pound, the weight in grams of 80.2 cc. is the same as the weight in pounds of one bushel. One-twentieth of this volume, or 4.01 cc., would be a suitable volume for measuring the grain from individual plants, and at the same time provide a simple conversion factor by which the weight of 4.01 cc. of grain, in grams, could be expressed directly in pounds per bushel.

A 25 cc. graduate, cut off at the 4 cc. point, was used to measure the grain from individual plants. The weight of the 4 cc. of grain in grams, times twenty, gave a good approximation of the weight per bushel in pounds. The grain was poured into the measure from a coin envelope, pressed lightly with the thumb, and then levelled off with a scalpel drawn across the top of the measure.

In order to compare the accuracy of the 4 cc. measure with the pint weight-per-bushel measure, weights per measured bushel were determined by both methods for 184 samples, including 69 varieties, of spring wheat; and 59 samples, including 46 varieties, of winter wheat. All of these samples were

taken from the regular varietal plots at Edmonton. One determination was made with the pint measure and four with the 4 cc. measure. In the latter case the average of the four determinations was used as the value for the weight of the sample. The probable error for the quadruplicated 4 cc. determinations for spring wheat was $\pm 0.28\%$, and for the winter wheat samples it was $\pm 0.34\%$.

The range of the spring wheats in pounds per measured bushel for the 4 cc. measure was from 60.5 to 68.5 lb., while the range for the same samples in the pint measure was from 59.0 to 67.0 lb. The correlation coefficient calculated from the weights obtained on the 184 samples of spring wheat by these two methods was $+0.947 \pm 0.005$ (Table I). The range of the

TABLE I

CORRELATION TABLE FOR 184 SAMPLES OF SPRING WHEAT, SHOWING THE ASSOCIATION BETWEEN THE TEST WEIGHT PER BUSHEL, OBTAINED BY THE PINT WEIGHT PER BUSHEL MEASURE AND THE 4 CC. GRADUATE MEASURE

Test weight per bushel by the 4 cc. graduate measure (y) \ Test weight per bushel by the pint measure (x)	59.0	59.5	60.0	60.5	61.0	61.5	62.0	62.5	63.0	63.5	64.0	64.5	65.0	65.5	66.0	66.5	67.0	f
60.5	1																	1
61.0		1																1
61.5	1																	1
62.0																		0
62.5			5		1													6
63.0							2	1										3
63.5					1		1											2
64.0							2	3	1									6
64.5						1	5	3	6	3	1		1					20
65.0								2	5	4	8		3					22
65.5								2	4	9	7	7	1					30
66.0									2	4	7	5	5	3				26
66.5										1	5	6	9		2			23
67.0											1	6	6	5	3	1		22
67.5												2	6	2	4		1	15
68.0															2			2
68.5															2		2	4
																		184
f_x	2	1	5	0	2	1	10	11	18	21	29	26	31	10	13	1	3	

$r = 0.947 \pm 0.005$

winter wheats in pounds per measured bushel for the 4 cc. measure was from 62.5 to 68.0 lb., while the range for the same samples in the pint measure was from 60.5 to 67.0 lb. The correlation coefficient calculated from the weights obtained on the same 59 samples of winter wheat by these two methods was $+0.834 \pm 0.027$.

In both of the above comparisons, it will be noted that the weights obtained with the 4 cc. measure are slightly higher than those obtained with the pint measure. The differences are so small that when placed on a percentage basis they are for all practical purposes really insignificant.

For the spring and winter wheat samples the regression equations of weight per bushel by the pint apparatus on weight per bushel by the 4 cc. method are: $x = 0.933y + 2.23$ and $x = 0.939y + 2.56$ respectively, where x = weight per bushel by the pint apparatus and y = weight per bushel by the 4 cc. method. The standard errors of prediction of the pint result, using the 4 cc. results are: 0.04 lb. for the spring wheat samples and 0.12 lb. for the winter wheat samples. These equations show the relationship existing between the two methods for obtaining the weight per bushel, thus enabling the result of one method to be expressed in terms of the other.

The high degree of correlation in the relative weights per measured bushel of different wheat samples, obtained by the pint and 4 cc. measures indicates that the latter measure is sufficiently reliable to be a useful instrument in determining the weight per bushel of small samples.

Kernel plumpness was determined by three methods for 315 F_3, lines, from a cross between Selection I-28-60 and Milturum, grown on dry land at Brooks, Alberta, in 1932. The methods used were: (i) weight per thousand kernels, (ii) weight per 4 cc. converted to weight per measured bushel, and (iii) an estimated kernel plumpness. The latter was determined by estimating the average degree of plumpness of the kernels in each sample, and assigning it a value of from 1 to 10, 1 representing completely shrivelled, and 10 completely plump. Intermediate degrees of plumpness were assigned values lying between 1 and 10. Correlation coefficients between each pair of the three methods for determining kernel plumpness were calculated from the data obtained. These are given in Table II.

TABLE II

CORRELATION BETWEEN DIFFERENT METHODS OF DETERMINING KERNEL PLUMPNESS IN 315 F_3 LINES, FROM A CROSS BETWEEN SELECTION I-28-60 AND MILTURUM

Variables correlated	r
Weight per thousand kernels and weight per bushel (4 cc.)	$+0.360 \pm 0.033$
Weight per thousand kernels and kernel plumpness	$+0.478 \pm 0.034$
Weight per bushel (4 cc.) and kernel plumpness	$+0.872 \pm 0.011$

There is a fair degree of correlation indicated when weight per thousand kernels is correlated with weight per bushel, $+0.360 \pm 0.033$; and with kernel plumpness $+0.478 \pm 0.034$. These results are, in general, in agreement with those reported by Bridgford and Hayes (1), Clark and Smith (2) and Waldron (3). A high degree of correlation was obtained when weight per bushel, as obtained with the 4 cc. graduate measure, was correlated with an estimated kernel plumpness, $+0.872 \pm 0.011$. These two methods appear to be the most reliable for determining the degree of shrivelling of wheat kernels in small samples.

Conclusions

When the plumpness of a large number of samples is to be determined, and an experienced person is doing the work, it would appear that the method of estimating the plumpness index on a scale of 1–10 would be the most useful. When the plumpness of a limited number of small samples is to be determined, and an accurate note is desired, the weight per measured bushel, as determined by the 4 cc. graduate, would appear to be the most useful. This method should be especially useful in experiments dealing with small samples harvested from pot cultures, in nutritional and disease studies as well as in plant breeding material. Weight per thousand kernels lacks reliability in comparisons when varieties and strains of different kernel size are involved, and is too laborious and expensive when thousands of determinations are to be made.

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THE ESTIMATED NUMBER OF NEMAS IN THE SOILS OF MANITOBA[1]

By E. H. J. Marchant[2]

Abstract

Samples of soil, taken from 29 different sections of the arable portions of Manitoba, have been analyzed. Twelve species of eight genera of nematodes have been identified. No species of the genus *Heterodera* were found.

An endeavor has been made, first, to determine the approximate degree of infestation, and second, to correlate such factors as hydrogen ion concentration, moisture equivalent, and organic matter content with the nema counts.

The nematode population is somewhat higher in the soils of Manitoba than in those of many parts of the United States, but considerably lower than in those of North China.

The number of species seems to be limited.

The degree of infestation appears to be negatively affected by either hydrogen ion concentration or moisture equivalent, but is decidedly influenced by organic matter content.

Introduction

Despite the fact that the soil Nematoda are almost world-wide in distribution, and that they are of both economic importance and scientific interest, they have attracted the attention of comparatively few investigators. So far as the author has been able to ascertain, the nemas inhabiting Manitoba soils have, up to the present, remained unstudied.

In determining the importance of the soil nematodes, their numerical significance must first be ascertained. The figures obtained are considered by most authors to be a minimum, due to the fact that, among the many difficulties experienced in compiling a census, complete isolation is, as yet, technically impossible, and secondly, to the uneven distribution of the nematodes over a wide area. (Steiner (17).)

The samples of soil, cultivated and uncultivated, taken from 29 districts of the arable portions of Manitoba, for this survey, were of the following genetic types:—meadow-prairie, podsol, reclaimed-meadow, podsolic chernozem, degraded chernozem, meadow, peat, sandy chernozem, alkalinized meadow-prairie, river terrace (immature), woodland invasion of prairie, and river flood plain (immature)

Technique

All samples were taken at a uniform depth of 6 in. (15.2 cm.) and obtained by the use of a metal cylinder, having an internal diameter of 72.1 mm. (one one-millionth of an acre (25)), and a length of 12 in. Four samples were collected from each area, and the final census, in each case, was determined by averaging these.

[1] *Contribution from the Department of Zoology, University of Manitoba, Winnipeg, Manitoba, Canada. This paper is constructed from a thesis presented to the Committee on Post-graduate Studies, University of Manitoba, April, 1934, in partial fulfilment of the requirements for the M.Sc. degree.*

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Isolation of the nematodes was accomplished by means of the Baerman apparatus, as used by Brown (4, 5) and described by Cort (9), with slight modifications. They were counted into a receptacle containing clean distilled water.

For the purpose of identification it was found best to examine the living organisms. However, when this was inconvenient, the following methods of fixing and preserving gave satisfactory results.

The specimens were relaxed and killed by heating in a hollow-ground slide containing a few drops of distilled water. Fixing was carried out by the use of Ditlevson's fixative, having the following composition: 6 parts formalin, 40 parts distilled water, 20 parts 90% alcohol, and 1 part acetic acid. From this the nematodes were transferred to a preservative composed of 68% of distilled water, 30% of 90% alcohol, and 2% of glycerin, in which they were left, unsealed, until water and alcohol had evaporated, leaving them finally in a slightly watery glycerin.

Table I shows the approximate number of nemas in the top 6 in. of an acre in each of the various soil types.

TABLE I

APPROXIMATE NUMBER OF NEMAS IN THE VARIOUS SOIL TYPES

Sample No.	Soil type	Soil texture	Approximate No. in the top 6 in. of an acre
1	Barnyard	Clay	6,250,500,000
2	Degraded chernozem	Silty clay	970,100,000
3	Chernozem	Silty clay	1,990,600,000
4	Garden	Silty clay loam	607,900,000
5	River terrace	Fine sandy loam	281,200,000
6	Meadow-prairie	Silty clay	4,980,000,000
7	Podzol	Fine sand	145,100,000
8	Reclaimed meadow	Silt loam	400,600,000
9	Podzol	Silty clay	878,900,000
10	Woodland invasion of prairie	Fine sandy loam	580,100,000
11	Podzolic	Silty clay loam	460,300,000
12	Chernozem	Heavy silty clay	634,600,000
13	Degraded chernozem	Clay	890,500,000
14	Garden	Silty clay	435,400,000
15	Peat	Muck	8,800,000,000
16	Degraded chernozem	Clay loam	2,200,000,000
17	Meadow	Silty clay loam	1,395,000,000
18	Degraded chernozem	H.V.F. sandy loam	643,500,000
19	Alkalinized meadow-prairie	Clay	480,600,000
20	Recent deposit	Silty clay	226,500,000
21	Meadow	Clay	262,800,000
22	Immature soil	Fine sandy loam	127,300,000
23	Sandy chernozem	L.V.F. sand	252,700,000
24	Chernozem	Fine sandy loam	325,100,000
25	Meadow-prairie	Clay	308,100,000
26	River flood plain	Silty clay loam	291,700,000
27	Chernozem	Silt loam	562,400,000
28	Meadow-prairie	Clay	2,790,000,000
29	Peat	Carr	*2,450

**One sample of 100 gm. only.*

TABLE II

MINIMUM NUMBER OF NEMAS PER ACRE IN VARIOUS FIELDS IN THE UNITED STATES

	Minimum number of nematodes per acre, top 6 in.
From Missouri corn field	648,000,000
North Carolina field	242,400,000
New Jersey field	129,600,000
Rhode Island field	610,800,000
New Hampshire field	99,600,000
Minnesota field	121,200,000
Vermont field	580,000,000
Kansas field	278,400,000

A census by Cobb (8) of fields in several of the United States is given in Table II, and is of interest for comparative purposes.

The results obtained would seem to indicate that the nematode population is somewhat higher, in the soils of Manitoba, than in those of many parts of the United States, but considerably lower than in those of North China (4, 5).

Taxonomy

The identification of the nematodes was facilitated by referring to descriptions or illustrations by the following authors: Baylis and Daubney (1), Cobb (6, 7, 8), Imms (10), Imperial Bureau of Agricultural Parasitology (11, 12), Micoletzky (13), Potts (14), Russell (15), Steiner (17, 18, 19, 20), Thorne (*21, 22, 23, 24*) and Waksman (*25*).

This survey yielded 12 species of 8 genera, and since they have all been previously described in detail by other investigators, the author has considered it necessary only to classify the species briefly, as follows:—

Nematoda

Fam. Rhabditidae
  Subfam. Rhabditinae
    Gen. *Rhabditis* (Dujardin) — *R. elegans* (Maupas); *R. cylindrica* (Cobb)
    Gen. *Cephalobus* (Bastian) — *C. subelongatus* (Cobb); *C. oxyuriodes* (DeMan)
    Gen. *Acrobeles* (Von Linstow) — *A. cervus* (Thorne)
  Subfam. Cylindrolaiminae
    Gen. *Plectus* (Bastian) — *P. cirratus* (Bastian)

Fam. Anguillulinidae
  Subfam. Anguillulinae
    Gen. *Anguillulina* (Gervais and Van Beneden) (Syn. *Tylenchus*) — *A. pratensis* (DeMan)
    Gen. *Ogma* (Southern) (Syn. *Hopolaimus*, Daday) (Syn. *Iota*, Cobb) — *O. octangulare* (Cobb)

Fam. Dorylaimidae
  Subfam. Dorylaiminae
    Gen. *Dorylaimus* (Dujardin) — *D. regius* (DeMan), *D. obtusicaudatus* (Bastian)

Fam. Oncholaimidae
  Subfam. Oncholaiminae
    Gen. *Mononchus* (Bastian) — *M. papillatus* (Bastian), *M. parvus* (DeMan)

Table III shows a list of the species and the various types of soil in which they were found.

TABLE III

SPECIES FOUND IN VARIOUS SOIL TYPES NUMBERED AS FOR TABLE I

Species	Sample number																												
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
Acrobeles cervus							*										*						*						
Mononchus papillatus													*		*							*							*
Dorylaimus regius		*	*		*	*	*	*	*	*	*		*	*	*	*	*	*		*	*	*	*	*	*	*	*		*
Dorylaimus obtusicaudatus		*	*		*		*		*		*		*	*	*		*				*	*	*				*		
Rhabditis elegans	*			*			*																				*	*	
Ogma octangulare																							*						
Cephalobus subelongatus	*	*	*	*					*			*		*	*			*	*			*	*			*	*		*
Rhabditis cylindrica	*														*							*							
Mononchus parvus							*																						
Cephalobus oxyuriodes	*			*	*							*		*	*							*							*
Anguillulina pratensis		*			*					*															*				
Plectus cirratus			*				*									*													

It is interesting to note (Table III) that, of the twelve species isolated, three, *Dorylaimus regius*, *Dorylaimus obtusicaudatus*, and *Cephalobus subelongatus*, occur much more frequently than do any of the others; also that two of the species, *Ogma octangulare*, and *Mononchus parvus*, each occur only once, the former in a grassland soil (sandy chernozem), the latter in a woodland soil (podsol).

The Relations Between Degree of Infestation and Moisture Equivalent, Organic Matter Content, and pH Value of the Soil

The soils used for these correlations were taken from the same sections as were those of the census. All determinations were made in duplicate and

averaged. Table IV lists the values obtained for moisture equivalent, organic matter, and pH of each of the soil types, numbered as in Table I.

TABLE IV

pH, ORGANIC MATTER, AND MOISTURE EQUIVALENT VALUES OF EACH OF THE SOIL TYPES

Sample No.	pH		Organic matter, %	Moisture equivalent, %	Sample No.	pH		Organic matter, %	Moisture equivalent, %
	H_2O	KCl				H_2O	KCl		
1	8.19	7.44	21.70	46.20	16	8.13	6.94	14.37	33.74
2	7.43	6.44	11.30	41.35	17	8.23	7.14	12.23	32.07
3	6.56	6.03	12.40	42.11	18	7.07	6.36	9.11	34.84
4	7.99	7.02	9.70	33.76	19	7.77	6.65	11.53	39.96
5	8.39	7.19	4.60	13.96	20	7.68	6.89	3.18	41.81
6	7.87	7.15	21.20	41.44	21	8.08	6.77	3.70	46.25
7	7.75	6.97	2.21	5.40	22	7.28	6.35	90.13	106.79
8	6.82	6.04	6.20	24.60	23	6.19	6.13	3.14	16.51
9	6.33	5.66	11.43	38.94	24	7.78	6.86	3.53	9.80
10	7.66	6.85	8.05	21.68	25	7.33	6.36	5.90	20.62
11	7.44	6.69	6.70	28.47	26	7.78	7.05	5.70	38.86
12	8.41	7.17	8.74	35.79	27	7.60	6.70	7.62	25.95
13	6.67	6.06	10.70	46.23	28	8.39	7.39	5.13	30.15
14	7.92	7.06	6.40	32.54	29	7.29	6.37	17.81	44.62
15	6.10	5.70	38.83	90.87					

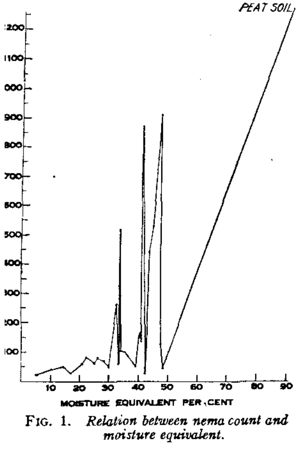

FIG. 1. *Relation between nema count and moisture equivalent.*

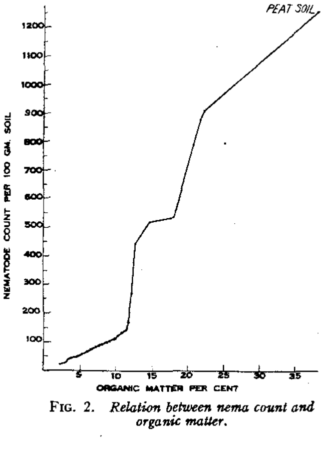

FIG. 2. *Relation between nema count and organic matter.*

Moisture Equivalent (M.E.)

This value was determined by the method described by Briggs and McLane (3). The percentage of moisture lost was determined on a dry soil basis; the percentage loss being the M.E. The relation between nema counts and moisture equivalent is shown in Fig. 1.

Organic Matter

This was determined volumetrically, by means of chromic acid (Schollenberger (16)). The percentage of organic matter was calculated on the basis of the dry weight of the soil. Fig. 2 shows the relation between organic matter and nematode count.

Hydrogen ion Concentration

The pH of the soils was determined, electrometrically, both in distilled water, and in normal potassium chloride solution, by means of the quinhydrone electrode (Biilman and Tovborg-Jensen (2)). The relation between pH and nema count is shown in Fig. 3.

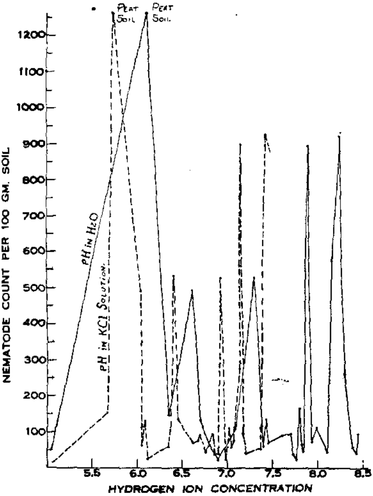

FIG. 3. *Relation between nema count and pH.*

Discussion

The results of this survey would indicate that while the nematode infestation is somewhat heavy, the number of species is limited. Extreme variations in temperature experienced in this province may perhaps be a contributory cause. This, however, can only be determined after the analysis of samples of soil, of a restricted area, taken at frequent intervals, and stratigraphically, over a period of one or more years.

Moisture equivalent (M.E.) appears to be a negative factor in affecting the nema population, although there seems to be a slight correlation below 15%. Above this the correlations are contradictory, except in the case of the peat soil. The writer is of the opinion that, in this instance, it is the high organic matter content which is the determining factor and not the M.E.

The organic matter content varied from 2–38% and appears to be closely related to the nematode counts, irrespective of soil type.

The hydrogen ion concentration (pH) of Manitoba soils, generally speaking, ranges from 5.50–8.00. Within these limits, no correlation with the number of organisms is apparent.

The fact that no species of *Heterodera* were isolated is not surprising, for in no country do they seem to be widespread, but rather confined to restricted areas. As far as the author has been able to ascertain, infestation has occurred only in two small districts, one near Chatham, Ontario, where *Heterodera*

schachtii Schmidt was found in a sugar beet field, the other near Humboldt, Saskatchewan, where a few wheat fields were mildly infected by a new species, *Heterodera punctata* Thorne. The writer obtained, through the kindness of Mr. R. C. Russell, Plant Pathologist, Dominion Laboratory of Plant Pathology, Saskatchewan, some specimens of preserved and dried wheat roots infected with the *H. punctata* Thorne. On these roots were a number of cysts, both in the white and in the brown or preservation stages. These were found to be pear-shaped rather than lemon-shaped as in the case of *Heterodera schachtii* Schmidt. Other differentiating characteristics of *H. punctata* Thorne, are that the female is smaller than that of *H. schachtii* Schmidt, and that it possesses a punctate cuticle.

Mr. G. Thorne is of the opinion that the *punctata* form of *Heterodera* is a native parasite of some indigenous plant or plants, and from the fact that it has selected wheat as its host, it seems probable that one or more of the native grasses will be found to be the original host.

No methods that are economically feasible have as yet been devised for the total eradication of *Heterodera* species. Up to the present, only crop rotation gives any worthwhile results. Stringent measures must also be taken to prevent the spread of infection. In the case of *H. punctata* Thorne, a system of crop rotation cannot be planned until its original host has been definitely determined.

During the period of this investigation, the crops, generally speaking, were in a poor condition as a result of drought and grasshopper injury, thus making it impossible to form any opinion as to the effect which the nematodes might have had upon their growth. Their effect on soil fertility is not yet fully known.

For the purpose of observing the predatory feeding habits of *Mononchus papillatus* a few experiments were carried out in the laboratory by feeding both larval and adult specimens of other species of nematodes. In every instance the adults were able to throw off the *Mononchus*, but in the case of the larvae, the body contents were first sucked out, and the cuticle then swallowed, with one exception. In this case a Rhabdite larva was swallowed whole. After being kept for ten weeks in water containing a few minute particles of soil, protozoa and other micro-organisms, but no nemas, they showed no signs of excessive hunger when offered larvae.

Many of the nematodes encyst whenever soil conditions become unfavorable, and in some instances, after having remained in this state for ten and even twenty-five years, they have become active again, when suitable conditions were restored. The author obtained from the Soils Department, University of Manitoba, samples of soil which had stood in the laboratory from ten to twelve years. Analysis of these however, gave negative results.

Acknowledgments

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THE RENEWAL AND REPLACEMENT OF THE STYLETS OF SUCKING INSECTS DURING EACH STADIUM, AND THE METHOD OF PENETRATION[1]

By A. D. Heriot[2]

Abstract

In a study of the mouth parts of the scale and aphis, certain conclusions corresponding to those of Hermann Weber were independently drawn. While several phases of the subject need clarifying, it is definitely established that the retort-shaped organs at the bases of the stylets are masses of hypodermal cells constituting deep invaginations of the integument. Within these invaginations, which lengthen out in a circular manner during each stadium, new stylets are built up for each successive instar. In those members of the Hemiptera in which the head is deflected, the four stylets are separately coiled in the cephalic region and at a definite stage during each ecdysis the new stylets pass down to take the place of the old which are discarded at the moult.

The manner of this renewal and replacement of the stylets appears to be, in many respects, unique. But, inasmuch as the stylets are simple hollow chitinous structures, they may be said histologically to bear a closer resemblance to other cuticular processes than they do to the more complex organs arising from imaginal buds during metamorphosis. An analogy to the renewal of the stylets is presented in the development of the spines and hairs of the body. The methods whereby the coiled stylets are expelled from the head are discussed.

The action of the protractor muscles in the aphis and scale is altogether inadequate to explain the means of penetration into the compact tissues of woody growths. More especially is this the case where the stylets are much longer than the labium, and are, moreover, looped within the body and thus neutralize the effect of muscular action at the base. New facts are advanced to supplement those already known, and it is submitted that the stylets are propelled forward by successive short holds contrived by the joint action of the labrum and labium. In the aphis the main function of the stylet muscles is directional control of the tips, enabling a short stylet to explore a large area. The absence of this control in the scale is compensated for by the provision of longer stylets. Directional control permits of selective feeding, giving rise to a sense of taste. This is manifest in specific types of injury to the host plant and also by a modification in the insect of accessory structures of the mouth.

Introduction

In the spring of 1928, investigations were commenced in the Entomological Laboratory at Vernon, British Columbia, to determine the part played by the woolly aphis of apple in facilitating the spread of canker. These studies were pursued over a period of several years, and the morphology and mechanism of the mouth parts of the aphis naturally received considerable attention.

The mouth parts of the woolly aphis had already undergone considerable study by other workers, most notably by J. Davidson (3) in England and A. C. Baker (1) in the U.S.A. In fact, there is no aphis on which there is a more extensive literature, with regard to both its external and internal anatomy, than *Eriosoma lanigerum* Haus. Nevertheless, such interesting features as the renewal and replacement of the stylets at the moult and the method whereby these delicate structures are inserted into the hard tissues of the host-plant have until recently been barely touched upon.

[1] *Original manuscript received May 3, 1934.*
Contribution from Dominion Entomological Branch, Department of Agriculture, Ottawa, Canada.
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Considerable information was gathered on these points in 1929 and 1930 when, in addition to the aphis, the scales and leaf-hoppers with similar deflected heads were examined for the purpose of obtaining comparative data. It was discovered that at each moult a new and more efficient set of stylets, which previously were coiled within the head, became available. This fact was alluded to by the writer (6) in a paper read before the British Columbia Entomological Society, and aroused considerable interest, being regarded as something new and therefore requiring further substantiation than could at that time be provided.

In the previous year, 1930, Hermann Weber's "Biologie der Hemipteren", published in Berlin, demonstrated, with much original illustration, the same interesting point and extended its application to the whole of the Hemiptera. While Weber is referred to by Imms (8) in his "Recent Advances in Entomology" (1931) it is evident that, when discussing the cephalic appendages, Imms had no knowledge of the "Biologie der Hemipteren" which deals so effectively with recent discoveries on this point.

This remarkable monograph came before the writer in June, 1933, through the courtesy of Prof. G. J. Spencer of the University of British Columbia, who had taken a great interest in another paper which was read before the Society, March, 1933, and which is now presented in a revised form.

I. Renewal and Replacement of the Stylets during each Stadium

In his text book, 1930, Imms (7) on summing up what was then known with regard to the mouth parts of the Hemiptera, refers to the oval areas of tissue at the enlarged proximal ends of both pairs of stylets as "the retort-shaped organs whose function is problematical" (Fig. 1). Davidson (2) was probably the first to draw attention to these peculiar organs in the embryo of *Eriosoma lanigerum* Haus. without appreciating their significance.

It is now established that these "retort-shaped organs" are masses of hypodermal cells which are engaged in building up new stylets to replace in due time the stylets in use which will subsequently be discarded at the moult.

In examining the mouth parts it was very soon found that the scale insects lend themselves more readily to microscopic study than the aphids. The scales such as *Aspidiotus ostreaeformis* Curtis are very thin and transparent. They are unable to move out of the field of vision under a high power when examined alive, and their mouth parts can thus be observed in action.

The particular problems that this paper endeavors to elucidate have always been regarded as of peculiar and special interest in the biology of the scale insects. Imms (7) sets out the nature of the problem as follows: "In Coccidae, the rostrum (labium) is very short and the stylets extremely long and the mechanism by which the latter are inserted into the plant and afterwards withdrawn and looped within the body is difficult to conceive." We are not concerned at the moment with the method of insertion, which will be dealt

with later, but more particularly with the idea of a withdrawal of the stylets which is conveyed in this sentence. This implies that the accepted opinion arrived at in 1930 was that the same set of stylets served the insect throughout the course of its three stadiums. There are several reasons for this idea. First, there is the inordinate and, to all appearances, unnecessary length of the stylets of the nymph which will be discussed later. Second, the fact that the stylets are never found with the exoskeleton of the other mouth parts attached to the cast skin, as in the case of the other Homoptera, and finally, there is the ease with which the scales can be lifted from the bark at the time of moulting (4). The last is entirely due to the fact that the old stylets embedded in the bark are more easily separated from the insect at the approach of the moult than they are from the host plant.

Our observations certify that no withdrawal of any kind takes place once the stylets are inserted into the bark. The old stylets are broken off during the struggles incurred in moulting. Sections through the bark of scale-fed twigs show the cast-off stylets of the two instars and the adult in the relative positions that these would be expected to assume when allowance is made for the

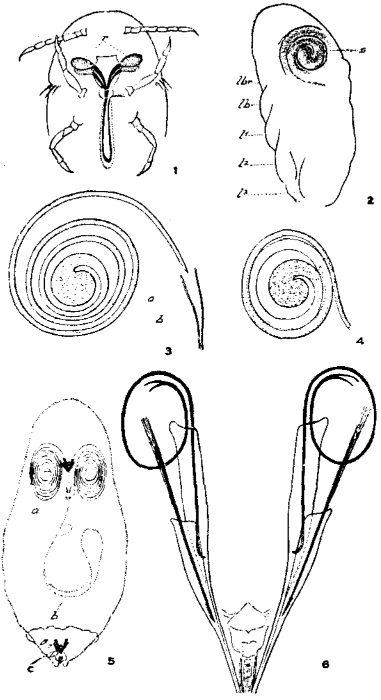

FIG. 1. *Coccus hesperidum: Figure adapted from Berlese and given by Imms. r, retort-shaped organs.* FIG. 2. *Section through embryo of Eriosoma lanigera. s, coiled stylet; lbr, labrum; lb, labium;* $l^{1\text{-}3}$, *legs.* FIG. 3. *Coiling stylet of Aspidiotus ostreaeformis from section. a, new stylet; b, old stylet.* FIG. 4. *Stylet coil dissected out of Eriosoma lanigera.* FIG. 5. *Lepidosaphes ulmi at second moult. a, stylets before moult; b, stylets after moult; c, moulted mouth parts in crumpled skin at posterior end of exuvia.* FIG. 6. *New and old stylets of Typhlocyba rosae showing coiled maxillary and straight mandibular stylets.*

slight movement that accompanies the insect's efforts to free itself from the exuvia. The stylets of each instar can readily be distinguished in these sections both by their respective diameters and by the different depths of their penetration.

Since these broad facts were known in 1929, considerable evidence has accumulated which allows for a more detailed description of what actually occurs at birth and at the moult with regard to the building up, renewal, and replacement of the stylets.

An examination of the embryo of any aphis or scale, shortly before birth, will show the long maxillary and mandibular stylets separately coiled in the head as in Fig. 2. During parturition or emergence from the egg, as the case may be, the separate stylets travel down from the head, their points converging to meet at the base of the labrum. Here the stylets coalesce and thence proceed as a single piercing and sucking organ to enter the labium.

The labium of the aphis is generally sufficiently long to accommodate the length of the stylets. In the scale, however, this is not the case, and only a small part of the stylets can find room in the very abbreviated labium. Hence all except the tips of the long stylets are, as Imms (7) expresses it, "looped and coiled upon themselves into the backwardly directed pocket (the crumena)."

This passing of the coiled stylets in the head to the labium and then to the crumena always takes place in *Lepidosaphes ulmi* L. at a definite stage of eclosion from the egg. This occurs before the head has freed itself from the egg envelope. Transitional stages are difficult to procure because, when once started, the movement seems to be very rapid. Only among large numbers of hatching nymphs have cases been observed with part of the stylets still in the head and part beginning to loop in the crumena. In the majority of cases, where the normal process is checked by examination media, the whole of the stylets pass out prematurely through the labium instead of folding back into the pocket provided for them.

When the stylets are in place, the invagination of hypodermal cells which brought them into being shrinks into a more or less oval mass of tissue around the bases of the stylets in the head, forming the retort-shaped organs. A small fold of this tissue extends like the finger of a glove into the mouth of each retort. At the apex of this fold the tip of the new stylet for the second instar begins to form. As the new stylet is laid down from the tip, the oval mass of tissue bunched at the mouth of the retort takes on a tubular form and lengthens out in a circular manner to form subsequently a coil resembling the curled frond of a fern (Fig. 3). In the core of this curling stem the new stylet is gradually built up, its relative proportions to the surrounding tissue of hypodermal cells being much the same as those of the lead to the wood of a cedar pencil.

As the long stylets proceed to be built up from the tip, the surrounding tissue retreats further and further towards the unfinished proximal end, increasing the number of convolutions in the coil, as it manufactures the

stylet. On completion of the requisite length, the creative cells bunch up at its base, later to insert another finger-like fold into the mouth of the new retort in the centre of the coil. This small fold, in turn, will subsequently furnish the adult with its stylet.

The scale has two or three weeks in which to lay down the long stylets for the succeeding instar in the manner described. On the other hand the aphis with its much shorter stylets accomplishes this feat in three or four days (Fig. 4). In *Typhlocyba rosae*, where the maxillary are longer than the mandibular stylets, the former are coiled while the latter find plenty of room in the buccal cavity of the head to develop without coiling (Fig. 6).

The new stylets of the second instar of *Lepidosaphes ulmi* L. are half as big again in both diameter and length as those of the first instar, which they supersede. At a definite stage of the moult, soon after the ventral skin has ruptured, the new stylets pass, as did those of the first instar, from the head to the body. The same quick transition from four separate coils in the head to a single sucking organ gracefully looped in the crumena attends the second and final moult (Fig. 5).

It may be of interest here to review briefly the steps that enquiry took in exploring this new ground. There was first the early discovery that the stylets of the scale were invariably coiled in the head previous to the moult, which at first suggested that the stylets were withdrawn into the head while the crumena was being renewed. The fact was patent, however, that the stylets of both the aphids and leafhoppers were cast off with the moulted skin, and it seemed most unlikely that the scale should be an exception to so general a rule. The finding of the cast-off stylets in the bark and the further fact that the formation of new stylets could be discerned when the old mouth parts were still functioning, led to an examination of the aphis and leafhoppers. This revealed the same condition of coiled stylets within the head in various stages of development. The serrated tip of the mandibular stylet of *Typhlocyba rosae* L. could unmistakably be detected in the retort of the mandibular stylet in use (Fig. 6).

The question arose as to whether this development and replacement of the stylets was something unique, or whether this method was duplicated in other structures at the moult. As it was quite out of the question that such purely chitinous hollow structures, without any matrix, could grow and be moulted like the other appendages, it seemed likely that the stylets, which are sometimes called the feeding bristles or setae, might be renewed as are many of the cuticular hairs of the body. These also become greater in length at each moult. The tubercles of the new hairs arise beneath the tubercles of the old, but the new hair itself lies compressed beneath the old skin and becomes erect when the body has dried after moulting.

A glance at Fig. 7, showing a multicellular spinal outgrowth *a*, and a single-celled seta *b*, will permit of the conception of a multicellular setal ingrowth *c*, illustrating the formation of a stylet. In this illustration the multicellular spine would correspond to the formation of the mandible of a biting insect.

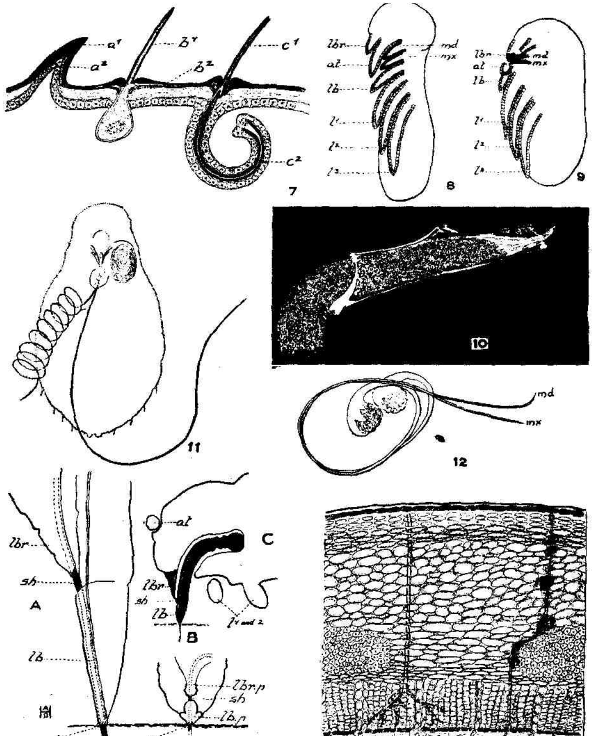

FIG. 7. a^1, a^2, *multicellular spinal outgrowth;* a^1, *old part,* a^2, *new part.* b^1, b^2, *unicellular setal outgrowth;* b^1, *old part,* b^2, *new part.* c^1, c^2, *multicellular setal ingrowth;* c^1, *old part,* c^2, *new part.* FIG. 8. *Embryo of biting insect. md, mandible; mx, maxilla; lbr, labrum at antenna; lb, labium;* l^{1-3}, *legs.* FIG. 9. *Embryo of scale; legend as in Fig. 8.* FIG. 10. *Broken stylet retort of Typhlocyba rosae showing tip of new stylet protruding beyond break with ligament.* FIG. 11. *Lepidosaphes ulmi; mandibular stylet issuing prematurely like a spring in corkscrew fashion.* FIG. 12. *Stylets of Lygus pratensis dissected from thorax.* FIG. 13. *Method of penetration. A, Eriosoma lanigera; B, Lepidosaphes ulmi; C, Eriosoma lanigera, section showing labium retracted into body. sh, short hold on stylet; t, thrust; lbr, labrum; lbr p, process at tip of labrum; at, antenna;* l^1 *and* l^2, *legs.* FIG. 14. *Stylet paths; that of Eriosoma lanigera on left, showing diversions by the tips and no injury to cortical cells; that of Lepidosaphes ulmi on right, showing absence of diversions and injury to cortical cells. Injury to phloem tissues not shown. (Diagrammatic in part.)*

Both the mixillary and mandibular stylets arise between the labrum and the labium as do their counterparts in biting insects. Fig. 8 shows the profile of the embryo of a biting insect with the outgrowths of maxilla and mandible, and Fig. 9 the profile of a scale embryo where the absence of projecting mouth parts must be accounted for by corresponding ingrowths.

Weber (9) gives a drawing, after Seidel, of the embryo of *Pyrrochoris apterus* Fall showing small projections which later become sunk into the head to form the first retorts out of which the stylets are built up. Observations with regard to the scale indicate that the long stylets are well advanced at the embryonic stage depicted by Seidel. When the embryo of the scale has gone through its reversion and the amnion has broken, the stylets are plainly visible. The writer cannot throw much light on the embryological development of the stylets except to maintain that the original invaginations must occur long before the reversion of the embryo, and in the deflected heads of the aphis and scale probably arise within a fold that separates the epipharynx from the hypopharynx.

There will be less cause for controversy in discussing the manner in which the stylets are drawn out or expelled from the head. Weber shows that the tips of the stylets are sometimes fastened to the inner side of the retort by a short ligament, and in the case of *Aradus* sp., to the extreme outer edge.

As the old stylets are cast off, the new stylets are drawn into place. This is corroborated in Fig. 10 which shows the broken retort of the maxilla of *Typhlocyba rosae* L. with a ligament at the tip of the new stylet protruding beyond the break.

No similar attachment has been discerned in *Eriosoma lanigerum* where the tips of the new stylets intrude into the retorts of the old, but as the old skin is cast away it is likely that a similar method of drawing out the stylets prevails.

In the case of the scale the skin is not cast away at the moult, the hard dorsal exuvia being only loosened and the ventral skin containing the mouth parts merely pushed down to its posterior end (Fig. 5). The new stylets, moreover, come down and are looped in the crumena soon after the ventral skin has broken at the anterior end. Some expelling force in this case seems to be essential. As a possible clue in this direction Fig. 11 illustrates a case where, in the course of mounting a moulting scale, the stylets have escaped prematurely through the labium instead of bending back into the crumena. One of the mandibular stylets is seen to have emerged in corkscrew fashion which suggests that the coiled stylets, as they mature, acquire the nature of a spring. In addition it can be shown that considerable pressure is exerted on the head by the narrowness of the exuvia. At an earlier stage the coils are conspicuously rounded, but at the approach of the moult they assume a distinctly oval form due to the compression of the head. Likewise the head of the young woolly aphis during parturition is held for a considerable time, and any pressure on the coils, which may at this stage have developed a tendency to straighten out, might initiate their expulsion. Once started, the labrum could then come into play in assisting their exit from the head.

As previously intimated, these studies have been confined to a few species where the head is deflected. Weber (9) shows that where the head is normal the stylets are coiled in the thorax. This is verified in Fig. 12, which shows the coiled stylets for the prospective adult dissected out of the thorax of the pupal instar of *Lygus pratensis* L.

II. Penetration of the Plant Tissues

The foregoing attempt to describe adequately the renewal and replacement of the stylets at the moult will assist in throwing light on the mechanical means whereby these slender structures are inserted into hard tissues.

Imms (7) says: "The problem which requires solution is the method by which long, slender and pointed stylets can be forced to the requisite depth into the tissues of a plant. In those cases where the stylets are but little longer than the labium, it has usually been explained that the action of the protractor muscles applied at the bases of the stylets, forces the latter into the plant, and that they are guided by the labrum and the grooved labium in their course."

No problem is presented in the case of the tarnished plant bug *Lygus pratensis* L. The penetration of soft tissues such as a bud seems to require no mechanism other than the bending of the labium as the head is lowered to force the stout stylets into the soft tissue. The same may be said of the leaf hopper, where the operation of penetrating the loose mesophyll of a leaf can be readily comprehended. In both these cases the mandibles are sharply serrated and with the muscles at their base no doubt play an important part in tearing out a path for the maxillae.

It is not such a simple matter to explain the penetration through the compact cortex of woody growths by the much more delicate stylets of the woolly aphis. Under a hand lens the newly born aphis may be observed sensing out a suitable spot to engage on, by means of the sensory organs at the tip of its long labium. The mandibular stylets are not serrated, and while there is proof that these can be used to deflect the tip of the maxillae in diverse directions after the stylets are inserted, their powers of penetration appear to be feeble. To make up for this deficiency the aphis is equipped with a comparatively much longer labrum than the tarnished plant bug and the leafhopper. This labrum is grooved along its length. A considerable portion of the stylet can thus be gripped with the whole weight of the head behind it. The long labium is also grooved and, moreover, can be retracted into the body, thus acting as a diminishing brace for the stylets. By successive short stages the labrum is brought into conjunction with the tip of the labium when the stylets are fully inserted.

When penetration by the scale is considered, an entirely different set of conditions has to be contended with. The scale stylets will not only penetrate the mature cortex of four- and five-year-old branches, but in new wood, where the layer of bark is thin, will enter the immature woody tissues. Cramped

beneath the shell-like exuvia the scale has little freedom of movement or choice of a suitable starting point and the tiny pimple of a labium is most inadequate as a brace. The muscles have little or no control over the tips of the stylets. This is accounted for by the long length of loosely looped stylets in the crumena. Even the possession of a labrum to make up for these deficiencies seems to have been denied the insect, according to other workers.

Evidently reasoning on somewhat similar lines Grove (5) and Berlese are referred to by Imms (7) as suggesting "that by means of blood pressure the apex of the labium becomes extended and consequently grips the stylets tightly after the manner of a pair of forceps. The projecting portions of the stylets would be forced a short distance into the plant tissues. This being accomplished, the internal pressure would be slackened, which would result in the grip being released and the labium would become slightly shorter, so that the apex would have traveled a short distance upwards along the stylets. The pressure being renewed, the grip is re-established and the stylets forced in a step further, and so on until the required amount of penetration may be accomplished."

This idea of successive short holds in propelling the stylets forward is strictly true, but these appear to be contrived by the joint action of the labrum and labium as in the aphis. Close examination of the labium will show that it is equipped with two vice-like processes, one being at the tip of the labium and the other at its base (Fig. 13B).

Between these two processes the tips of the stylets enter the labium. The hinder process is movable and is undoubtedly the tip of the labrum which in the scale enters the labium instead of being superimposed as in the aphis. Fig. 13 shows a short hold on their respective stylets by the labrum and labium of an aphis and a scale. The difference in the method of penetration is that the aphis retracts the long labium into the body and the scale protracts the long stylets out of the body.

The stylets of the aphis are nearly twice the diameter of those of the scale and may be assumed to be twice as stiff. Hence the shortness of the hold would be less and penetration correspondingly much more rapid. The short labrum of the scale, in spite of the pincer-like grip depicted in Fig. 13, has a much weaker hold on a more flexible stylet. This must necessitate a very short hold indeed, with consequently very slow penetration.

These inferences are borne out by sections showing the paths of both aphis and scale stylets through the same tissue. No damage to the cells of the cortex is noticeable in the straight path of the larger aphis stylet which pierces these to reach its objective in the region of the cambium. The stylet of the scale, on the other hand, no sooner enters the cortex than broken-down cells appear in its path, indicating that in its much slower progress the scale has to feed on the cell contents that the tips of the stylets invade (Fig. 14).

The same sections show a marked difference in the control of the stylets by the aphis and the scale. The tips of the aphis stylets make numerous diversions into the surrounding tissue after they are inserted. The length of these diversions corresponds closely to the depth of the buccal cavity in the head into which the stylets can be withdrawn. The mandibular stylets, working independently, are then able to manipulate the tips of the maxillae in new directions. These diversions appear to be entirely absent in the scale, undoubtedly owing to the looped condition of the stylets.

The action of penetration thus becomes more or less automatic in the scale. This was evidenced when a normal adult was turned over, showing that the tips of the stylets had met with some obstruction. The greater part of the stylets had been propelled out of the body without any portion entering the bark. Unlike the aphis the scale has no definite objective and appears satisfied with any tissue of its many host plants. Sections through the annual growth of apple reveal the scale stylets *in situ*. These are shown passing straight through the cortex, being turned aside by a bundle of pericycle fibres, continuing their course through the phloem, crossing the cambium, and invading the immature xylem. They are only prevented from further incursions by the mature fibres of the wood which deflect them into a course parallel with the cambium. Slow penetration and lack of directional control appear to be compensated for by the provision of long stylets.

Within the limited range of these observations, directional control of the stylets is accompanied by a discrimination as to host plants and the tissues attacked. Selective feeding gives rise to specific characters in various galls, by which the species responsible may be identified. A sense of taste is also clearly marked in the higher development of the gustatory organ of the epipharynx in the aphids that form galls. This organ is very reduced in the scale, but the salivary pump above it is correspondingly much enlarged to provide the power needed to force the saliva down the long duct.

In *Adelges cooleyi* Gillette we have a form that brings the aphis and scale into closer relationship. The summer form on Douglas fir is distinctly *Aleyrodid* in character. The first instar has stylets nearly as long as the scale and looped within the body. The labium is longer than that of the scale but the labrum is longer than the labium. The mechanism of the mouth parts is obscure but the rapidity with which the stylets are withdrawn into the body if the insect is disturbed indicates that the labrum plays an important part in penetration.

Acknowledgment

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THE ANALYSIS AND COMPOSITION OF THE FLESH OF THE DOMESTIC FOWL[1]

By R. Holcomb[2] and W. A. Maw[3]

Abstract

Determinations have been made of the moisture, fat, ash, and protein content of 34 chickens. The samples consisted of the skin, fat and flesh of the entire carcass. The methods of analysis used were: moisture, drying *in vacuo* at 50° C.; fat, hydrochloric acid digestion; ash, ignition at 700° C.; nitrogen, Gunning modification of the Kjeldahl; protein, by difference. The percentage composition has been found to be:

	Moisture	Fat	Ash	Protein	Nitrogen
Average of 32	65.33	13.99	0.89	19.79	3.298
Standard deviation	4.10	4.99	0.05	1.59	0.507
Maximum	71.81	28.92	0.98	22.33	3.528
Minimum	54.23	6.35	0.77	16.08	2.981

The fat content was found to be inversely proportional to the percentage of moisture, the correlation coefficient between the two being −0.962. A comparison was made of the hydrochloric acid digestion method and the Soxhlet extraction of fat, the former being found to be much superior in reliability of results and in economy of time.

Introduction

In connection with a nutritional study in progress in the Department of Poultry Husbandry of Macdonald College, analyses have been made of representative individuals from lots of chickens in fattening trials under confinement. The aim of the study was to determine the effect of the protein level upon the condition of finish in poultry under forced feeding in preparation for market. A general discussion of the problem and the results of the work up to the present will be given elsewhere. It was thought that in the meantime, a description might be given of the methods of chemical analysis which have been used, together with some observations on the data obtained.

It was decided at the start of the work to analyze the entire edible carcass of each bird rather than to select for examination a certain portion such as a particular muscle tissue. The sample, as received in the laboratory, consisted of the skin and attached fat, together with the muscular flesh (dark and light meat) which had been cleanly removed from the skeletons of those birds that had been chosen from the feeding lots for analysis. The abdominal fat was also included in the sample since the increase in the amount of external fat is apparently closely related to that of the abdominal fat. The constituents to be estimated were the moisture fat, ash and protein.

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Crude protein is customarily estimated by multiplying the percentage of nitrogen (Kjeldahl) by a factor. The nitrogen factor for muscular tissue is given as 6.25, for skin and connective tissue as 5.55, and for the nitrogenous bases as small as 3.34. As a consequence of these differences in the nitrogen content of the constituents of meat, and as the relative amounts of the different tissues would be different in the separate samples, it was decided to report the nitrogen content directly and to report the protein by the difference method as first suggested by Atwater (3).

Since meat is composed almost entirely of moisture, fat, ash and protein, the difference between 100 and the sum of the percentages of the three other constituents gives the percentage of crude protein. According to some (2) this figure has often more significance than N × 6.25. With data for the nitrogen content and for the protein by difference of the several birds analyzed, it was possible to compute the nitrogen in the protein fraction and to observe the influence of the nitrogen content of the feed upon this figure.

Description of Methods

Preparation of Sample

The sample as received was first chilled until time was available for work upon it. It was then cut into strips (about 1 in. wide) and put through a meat grinder with the coarse cutter attached. The grinding was repeated ten times, with thorough mixing after each passage through the grinder. As soon as the consistency would allow, the coarse cutter was replaced by one of medium size, usually after the fifth or sixth grinding. Passage of the sample through the grinder only three times was recommended by Wiley (10) and is still to be found in the methods of the A.O.A.C. (1). This may be sufficient for sampling separate tissues, but in the work being described, even after ten grindings individual tissues could be easily recognized. Owing to this inhomogeneity in the sample large weights of material had to be used for the separate determinations.

Moisture

Samples of about 20 to 30 gm. were rapidly weighed (to 0.005 gm.) into salve boxes of tinned metal, 3 × $\frac{3}{4}$ in., with tight fitting covers. About 90% of the moisture was removed at 50° C. *in vacuo* (four days) and the remainder at 105° C. at atmospheric pressure.

Ash

The moisture-free samples from the above determination, added to crucibles in successive small portions, were burned carbon-free at 700° C., the oxidation of the carbonized residues being assisted by small additions of nitric acid.

Fat

A modification of the method given by Buttenberg (4) was devised and found distinctly superior to the Soxhlet both in completeness of extraction and in quality of the extracted fat (see Discussion of Methods). Samples of about 10 to 15 gm. of the original material were rapidly weighed into

150-ml. Erlenmeyer flasks and covered with 25 ml. of concentrated hydrochloric acid. The flasks, loosely covered with small funnels, were left overnight on a boiling water bath. With the aid of hot water, the digested mass, now almost entirely liquid, was transferred in about equal portions to two Mojonnier extraction flasks. After cooling, each tube was extracted with four successive portions of a 1 : 1 mixture of ethyl and petroleum ethers, the Erlenmeyer also being rinsed with the ethers. The extracts were combined in a tared flask, the ether distilled off, and the residue dried at 100° C.

Nitrogen

Samples of about 20 to 30 gm. were digested with 100 ml. of concentrated sulphuric acid and 20 gm. of potassium sulphate-copper sulphate mixture. Five ml. of kerosene was added to prevent foaming. The resulting liquid was made up to 200 ml. with water and aliquots taken for the distillation.

Discussion of Methods

Preparation of Sample

The importance of the lack of homogeneity in the sample is shown by its effect upon the values of the mean deviations in the separate determinations, and the consequent precision of measurement. In Table I the mean deviations in the determination of moisture have been grouped according to size, and averages taken within these groups. The averages for the corresponding deviations in the determination of fat have also been taken. The direct proportionality between the two sets of averages would show that, since the lack of homogeneity is one of the most important sources of error common to both determinations, it is the most influential factor affecting their size.

TABLE I

Relation between deviations in moisture and fat

Group		Average of mean deviations	
Range	Number	Moisture	Fat
0.00 to 0.09	12	0.05	0.16
0.10 to 0.19	7	0.13	0.24
0.20 to 0.39	5	0.28	0.30
0.40 to 0.59	4	0.46	0.42
0.60 to 0.79	1	0.64	0.61
0.80 to 0.99	0	—	—
over 1.00	1	1.48	1.72
Left out	4		

Moisture

During the drying at 50° C. the meat retains its flesh color and shrinks to a transparent horn-like mass. The drying is at first more rapid than at higher temperatures, where color changes appear, associated with protein coagulation. The last portions of the moisture are safely and best removed at 100° C.

Fat

Digestion methods for fat in meats were recommended by Konig as early as 1914 (6) and also by Buttenberg (4), but American chemists (1) have adhered to the Soxhlet procedure, in some cases reluctantly (3). Preliminary experiments were made with the latter method both with samples dried at 105° C. in air, and upon those dried at 50° C. *in vacuo*, and with ethyl and

petroleum ethers as extractants. The extractors were heated by electric light bulbs, adjustable as to height, and broken porcelain was used in the flasks to prevent bumping. The fatty residues obtained were dark in color, like molasses, and had a disagreeable burnt odor. They would obviously be unsuited for the determination of physical constants. In contrast, the residues from the digestion method closely resembled hot-water-extracted chicken fat in color and odor and also in the absence of an ultra-violet fluorescence observed in the Soxhlet fats by use of a Callophane filter.

Comparisons of the results obtained by the two methods are given in Table II. It will be seen that, with the exception of the first three samples, the percentage of fat extracted is materially higher by the digestion than by the dry-extraction method. The average difference is 1.64% of the total weight and is equal to 12.0% of the average fat content of the samples. The maximum difference, 4.83% (sample 142), is about 25% of the fat content of the sample or about 32% of that indicated by the Soxhlet method. The average deviation of duplicates was practically the same in the two methods (0.30 and 0.32), showing that both have about the same degree of precision. The digestion method is to be recommended as it requires less time and less fragile apparatus, obviates the necessity of drying the sample and the danger of ignition of the extractant and finally, appears to give more trustworthy results.

TABLE II

Comparison of methods for fat

Sample No.	Soxhlet			Digestion	
	Dried at	Extractant	% Fat	% Fat	Difference
219	105°	Petrol. ether	13.28	11.33	−1.95
42	105°	Petrol. ether	9.44	9.26	−1.18
3141	105°	Petrol. ether	11.93	12.02	0.09
77	105°	Petrol. ether	5.54	6.35	0.81
3196	105°	Petrol. ether	8.96	9.80	0.84
4564	105°	Petrol. ether	12.43	13.99	1.56
3119	105°	Petrol. ether	12.84	14.92	2.08
3140	105°	Petrol. ether	12.73	16.25	3.52
417	105°	Petrol. ether	12.65	16.83	4.18
142	105°	Petrol. ether	14.75	19.58	4.83
1885	50°	Petrol. ether	13.37	16.61	3.24
		Ethyl ether	15.98		0.63

Protein

Atwater and others (2, 3, 4, 7) have pointed out that the conventional nitrogen factor, 6.25, does not properly apply to the mixture of proteins of meat. Some advocated the reporting of protein by difference and of nitrogen content as rational independent figures. The factors necessary to convert the nitrogen to protein as obtained by difference varies with the proportions of the various tissues in the sample. Reference to Table III shows that for

TABLE III

THE PERCENTAGE COMPOSITION OF CHICKENS (EDIBLE PORTION MINUS GIBLETS)

Sample	Ration	Moisture	Fat	Protein	Ash	Nitrogen	N in protein, %	Sample
14	II	66.24 ± 0.29	11.07 ± 0.15	21.75 ± 0.45	0.94 —	3.379 ± 0.002	15.54 ± 0.32	14
19	check	68.70 0.14	9.67 0.33	20.68 0.48	0.95 ± 0.02	3.332 0.099	16.11 0.61	19
42	III	70.27 0.08	9.26 0.04	19.56 0.15	0.91 —	3.432 0.010	17.54 0.14	42
66	check	71.14 1.48	10.23 0.72	17.68 2.20	0.95 —	3.256 0.021	18.42 2.29	66
77	V	71.81 0.09	6.35 0.10	20.86 0.20	0.98 0.01	3.521 0.016	16.88 0.18	77
103	check	60.43 ± 0.12	20.36 ± 0.76	18.33 ± 0.98	0.88 ± 0.10	3.141 ± 0.089	17.13 ± 1.04	
142	V	59.82 0.24	19.58 0.64	19.78 0.88	0.82 0.00	3.030 0.019	15.32 0.71	142
144	check	69.25 0.06	10.38 0.25	19.41 0.33	0.96 0.01	3.137 0.051	16.16 0.37	144
172	V	66.23 0.05	10.75 0.24	22.06 0.29	0.96 0.00	3.511 0.006	15.91 0.21	172
219	I	67.88 0.45	11.33 0.32	19.90 0.78	0.89 0.01	3.235 0.016	16.26 0.64	219
349	IV	62.15 ± 0.30	19.59 ± 0.59	17.44 ± 0.89	0.82 ± 0.00	—		349
358	III	60.56 0.03	18.94 0.55	19.69 0.59	0.81 0.01	—		358
417	IV	62.15 0.20	16.83 0.06	20.16 0.27	0.86 0.01	3.321 ± 0.006	16.47 ± 0.22	417
789	check	67.65 0.06	10.80 0.18	20.61 0.24	0.94 0.00	3.414 0.038	16.56 0.24	789
1051	check	65.10 0.09	13.13 0.33	20.89 0.42	0.88 0.00	3.201 0.042	15.32 0.31	1051
1085	IV	63.99 ± 0.01	13.91 ± 0.10	21.19 ± 0.12	0.91 ± 0.01	3.625 ± 0.059	17.11 ± 0.29	1085
1088	V	64.47 0.05	16.31 0.05	18.36 0.12	0.86 0.02	—		1088
1142	III	70.29 0.16	6.53 0.07	22.33 0.24	0.85 —	—		1142
1225	I	63.05 0.10	11.17 0.05	24.88 0.16	0.90 0.01	—		1225
1269	II	62.78 0.11	17.16 0.12	19.22 0.24	0.84 0.01	—		1269
1354	V	64.12 ± 0.01	15.14 ± 0.10	19.86 ± 0.12	0.88 ± 0.01	—		1354
1364	II	63.22 0.18	11.85 0.25	24.07 0.45	0.86 0.02	—		1364
1448	III	54.23 0.37	28.92 0.08	16.08 0.46	0.77 0.01	—		1448
1864	I	64.84 1.57	14.54 0.17	19.75 1.77	0.87 0.03	3.220 ± 0.031	16.30 ± 1.24	1864
1885	II	63.59 0.64	16.61 0.61	18.92 1.26	0.88 0.01	3.112 0.003	16.50 1.09	1885
2818	I	57.65 ± 0.12	24.13 ± 0.67	17.45 ± 0.79	0.77 ± 0.00	—		2818
3108	check	71.59 0.11	8.45 0.12	19.02 0.25	0.94 0.02	2.981 ± 0.065	15.67 ± 0.39	3108
3119	III	64.32 0.09	14.92 0.37	19.89 0.37	0.87 0.01	3.261 0.037	16.40 0.35	3119
3140	IV	61.88 0.03	16.25 0.07	20.93 0.11	0.94 0.01	3.528 0.064	16.85 0.32	3140
3141	II	66.65 0.45	12.02 0.79	20.43 1.45	0.90 0.01	3.122 0.036	15.28 0.92	3141
3148	IV	67.58 ± 0.51	10.85 ± 0.38	20.68 ± 0.89	0.89 ± 0.00	3.353 ± 0.018	16.21 ± 0.70	3148
3176	I	68.54 0.42	9.87 0.20	20.71 0.63	0.88 0.01	3.356 0.039	16.20 0.56	3176
3196	I	68.53 0.03	9.80 0.60	20.73 0.64	0.94 0.01	3.392 0.023	16.36 0.54	3196
4564	II	66.15 0.01	13.97 0.17	18.94 0.20	0.94 0.02	3.282 0.032	17.35 0.25	4564
Average (32)		65.33±4.10	13.99±4.99	19.79±1.59	0.89±0.05	3.298±0.51	16.41±0.70	

the samples of poultry meat here reported, it would vary from 5.43 (sample 66) to 6.53 (sample 142) and would average 6.094, corresponding to a nitrogen content of 16.42% in the protein.

The objection that, by estimating protein by difference, one is imposing upon this constituent the accumulated errors of the other three—fat, ash and moisture—is not, in our opinion, a very serious one. In the results reported in Table III the mean deviation of the protein by difference is 0.57 or 2.87% of the average protein content while that for nitrogen is 0.034 or 1.05% of the average nitrogen content.

Results

Composition

The composition of the 34 chickens analyzed is set out in Table III. The five rations referred to in the second column are described in Table IV,

TABLE IV

PERCENTAGE COMPOSITION OF THE RATIONS

Ration No.	Moisture	Fat	Fibre	N × 6.25	Ash	N-free extract
I	10.56	5.24	2.78	14.18	2.53	64.71
II	10.33	4.78	2.60	16.42	3.19	62.68
III	10.17	5.34	2.48	18.09	3.51	60.41
IV	10.36	5.40	2.27	19.64	4.60	57.73
V	10.18	5.72	2.13	22.69	5.79	53.49

although the discussion of the effect of these rations upon the composition will be given elsewhere. The percentage by weight of each constituent is supplemented by a figure representing the average deviations of the duplicate determinations from their mean. In studying the data with reference to the influence of the feeding upon the composition, as will be described elsewhere, this figure may serve as a rough measure of the value of the mean percentage. The mean deviations for the protein by difference are the sums of the deviations of the three other constituents. The figure given as the mean deviation for the percentage of nitrogen in the protein by difference has been calculated from the relation,

$$d_{NP} = \sqrt{\left(\frac{100d_N}{P}\right)^2 + \left(\frac{100d_P N}{P^2}\right)^2}$$

where N and P are the percentages of nitrogen and protein respectively, d_N and d_P are the corresponding deviations for these two percentages, and d_{NP} is the "precision measure" of the percentage of nitrogen in the protein.*

At the foot of the table is given the average composition of 32 birds (Nos. 1225 and 1364 have been excluded for reasons to be given below). The standard deviations of the groups from these averages are also given as a measure of the range of variation of each constituent.

* *The following texts have been used as guides in the statistical examination of the analytical data: Goodwin's Precision Measurements and Graphical Methods, New York, 1920; Tippett's Methods of Statistics, London, 1930; and Bowley's Elements of Statistics, 5th ed., London, 1926*

Although these chickens had been selected for the feeding experiment because of their uniformity, were of about the same age, and had been fed almost similarly in the trials, there is a wide range in their composition. The percentage of moisture ranges from 54.23 to 71.81 with an average of 65.33. The percentage of fat shows the same wide variation, ranging from 6.35 to 28.92 with an average of 13.99. The variation in the percentages of ash, protein, and nitrogen is much less, as shown by the relatively small standard deviations.

There appears to be a remarkable individuality in the composition of animals. This has been recognized (8) as one of the major difficulties in the study of animal response in nutritional investigations. As has been noted, even the percentage of nitrogen in the protein fraction, a ratio which one would imagine to be fairly constant for any animal species, shows a wide variation.

Relation between Constituents

During the progress of the analyses, it was noticed that there was an inverse proportion between the percentages of fat and moisture. A scatter diagram of this relation is shown in Fig. 1, each point representing the corresponding fat and moisture content of one bird. The linear regression equation, F = −1.170 M + 90.48, is drawn in to show the regular distribution over the range. Since the composition of birds No. 1225 and No. 1364 (marked by squares) appeared irregular, it was suspected that either the samples as received, or the birds themselves were abnormal in composition, or that some error had crept into the analysis. Two additional birds from the same feeding lots were analyzed to replace the two of doubtful composition. To measure the degree of association between the two constituents for the 32 pairs, the correlation coefficient was computed and found to be −0.962. This figure is sufficiently close to −1.0 to be considered as an almost perfect association. The two regression equations were found to be,

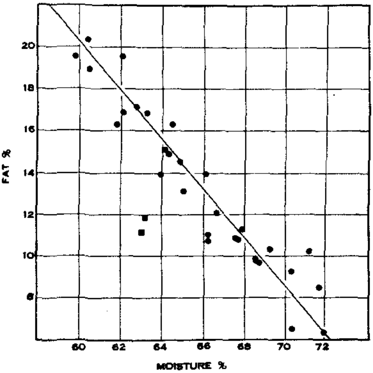

FIG. 1. *Relation between fat and moisture.*

$$F = -1.170 M + 90.48,$$

and

$$M = -0.791 F + 76.40,$$

the former giving the best value for the percentage fat calculated from any moisture content, and the latter the best value of the percentage of moisture calculated from any percentage of fat. Their value is shown as a guide in judging the worth of the results of the analyses of birds No. 1225 and No. 1364, the deviations in these cases being over four times the average deviation.

From a biological viewpoint this relation between fat and moisture is especially interesting as the fat in birds, for the most part, is laid down under the skin and within the abdomen, while the moisture is fairly evenly distributed through the muscular and connective tissue.

This relation between fat and moisture was pointed out by Wait (9) who concluded from his results that, "the fluctuations in the amount of fat follow almost exactly the fluctuations in the amount of water, - - - - -. This relation of fat and water holds true for nearly all kinds of flesh which have been analyzed."

A notable example of this relation is to be found in the work of Haecker (5), although it was neither noted nor discussed by him. From his data given for the percentage composition of steers, the correlation coefficient for the percentages of moisture and fat in the carcass is found to be -0.997, the regression equations corresponding to those given above, being,

$$F = -1.18\,M + 88.71,$$

and

$$M = -0.83\,F + 74.71.$$

The small differences in the constants in these two sets of equations is more than likely due to the natural differences in the composition of steers and chickens, although these unrelated animal species show the same relation between their fat and moisture contents. When additional analytical data are at hand, it will be interesting to see if this relationship is general in the animal kingdom.

TABLE V

RELATION BETWEEN MOISTURE AND ASH

Per cent ash	Average percentage moisture
0.77	55.94
0.81	60.56
0.82	60.98
0.84	62.78
0.85	70.29
0.86	63.31
0.87	64.85
0.88	64.36
0.89	67.73
0.90	66.65
0.91	67.13
0.94	68.03
0.95	68.92
0.96	67.74
0.98	71.81

There are no very definite relations between any of the other constituents. The correlation coefficient for the association of moisture and protein is $+0.426$, which, however, is too low to be considered significant from a practical point of view. The percentages of ash and moisture are only roughly proportional. The percentages of moisture corresponding to the different percentages of ash have been averaged and are shown in Table V. As the moisture content of the meat increases so also does the ash. The increase is far from regular but with the exclusion of three pairs (0.85, 0.89 and 0.96) a general parallelism is seen.

Acknowledgment

We take pleasure in acknowledging our indebtedness to Dr. J. F. Snell for many suggestions and for his continued interest in the work, and also to Dr. W. W. Stewart and Dr. Darol K. Froman for their kind assistance with portions of the mathematical treatment.

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INFLUENCE OF CYSTEINE ON THE PRODUCTION OF HAEMOTOXIN OF *CL. WELCHII*[1]

By J. H. Orr[2] and G. B. Reed[3]

Abstract

It has been shown that the addition of 0.1% or more of cysteine to Robertson's chopped meat media completely inhibits the production of haemotoxin by Cl. welchii and that H_2S has a similar effect. The effect is shown to be related to the metabolism of the organisms and not to a direct reaction with the formed toxin. The addition of these concentrations of cysteine was shown to have little effect upon the oxidation-reduction potential of sterile media but a marked effect upon the oxidation-reduction potential of cultures during the most active growth period. Beef muscle media low in cystine were shown to give a good yield and fish muscle relatively high in cystine was shown to give a poor yield of haemotoxin.

During the course of an investigation of the haemotoxin of *Cl. welchii*, Reed, Orr and Burleigh (6), Orr, Campbell and Reed (4), we have experienced much difficulty with variation in yield of haemotoxin from the same cultures.

The problem of the variability of the organisms has recently been discussed by Orr, Josephson, Baker and Reed (5). In the later work on toxin production the only cultures used have been those which have been so stabilized by selection that they fail to show variation as determined by colony structure and by agglutination reactions. But notwithstanding apparent uniformity of type in successive cultural generations, wide variation in haemotoxin yield has frequently been observed. It seemed apparent, therefore, that the irregularity was dependent upon lack of uniformity in the culture media or in some other environmental factors.

The amino acid content of the medium was shown by Davis and Ferry (2) and some others to have a conspicuous influence upon the yield of diphtheria toxin and recently Walbum and Reymann (9) have shown that the increase in amino nitrogen during the course of development of a culture of *Cl. welchii* runs parallel, up to a maximum amount, to the production of killing toxin. We therefore undertook an investigation of the influence of the amino acid content of the culture media upon the yield of haemotoxin.

Influence of Amino Acids on Haemotoxin Production

A series of flasks was arranged, each containing 100-cc. portions of Robertson's chopped-beef medium with 0.1% glucose. To each flask was added one of the following amino acids to a concentration of 0.1%: alanine, cysteine, cystine, tyrosine, histidine, tryptophane and glycine together with creatin and creatinine in the same concentration. The flasks were boiled, rapidly cooled, and inoculated with an S type toxin-producing strain of *Cl. welchii*,

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sealed with vaseline and incubated at 37° C. for 21–22 hr. After filtration the filtrates were titrated against washed rabbit red cells. This titration was carried out immediately with a minimum exposure to air, to reduce the possibility of oxidation of the toxin as such a reaction was shown by Neill (3) and ourselves (7) to be an important factor in the activity of haemotoxins.

Haemotoxin titrations were made by setting up serial dilutions of the toxic filtrates in freshly boiled saline to which washed rabbit red cells to 2% were added. After three hours' incubation, with frequent shaking, in a 37° C. water bath the maximum dilution of the toxin which caused haemolysis of half the cells was determined by matching the centrifuged reaction tubes against a color standard.

The results shown in Table I indicate clearly that two of the amino acids, cystine and cysteine, have a profound inhibitory effect upon the haemotoxin

TABLE I

TITRATION OF *Cl. welchii* HAEMOTOXINS PREPARED FROM MEDIA CONTAINING DIFFERENT AMINO ACIDS, CREATIN, AND CREATININE

Amino acid, 0.1%	Toxin concentration												
	$\frac{1}{20}$	$\frac{1}{40}$	$\frac{1}{60}$	$\frac{1}{80}$	$\frac{1}{100}$	$\frac{1}{200}$	$\frac{1}{400}$	$\frac{1}{600}$	$\frac{1}{800}$	$\frac{1}{1000}$	$\frac{1}{2000}$	$\frac{1}{4000}$	Control
Cysteine	+	−	−	−	−	−	−	−	−	−	−	−	−
Cystine	+	+	−	−	−	−	−	−	−	−	−	−	−
Alanine	+	+	+	+	+	+	+	+	+	±	−	−	−
Tyrosine	+	+	+	+	+	+	+	+	±	±	−	−	−
Histidine	+	+	+	+	+	+	+	+	+	+	−	−	−
Glycine	+	+	+	+	+	+	+	+	+	+	±	−	−
Tryptophane	+	+	+	+	+	+	+	±	−	−	−	−	−
Creatin	+	+	+	+	+	+	+	+	+	±	−	−	−
Creatinine	+	+	+	+	+	+	+	+	+	+	±	−	−
No addition	+	+	+	+	+	+	+	+	+	+	−	−	−

production by this organism and that the other amino acids tested are without effect in the concentration tested. The experiment has been repeated many times—the addition of 0.1% of cysteine to the chopped meat medium has never failed to inhibit haemotoxin formation. The fact that alanine, which has the same structure as cysteine except for the absence of the SH group, shows no inhibitory effect, suggests that the inhibition is due to the sulphur or the SH group. The difference in the solubility of cysteine and cystine probably accounts for the greater inhibitory activity of the former.

In order to determine the concentration of cysteine necessary to bring about inhibition, a series of flasks of chopped meat media containing decreasing concentrations of the cysteine, ranging from the 0.2 to 0.001% were inoculated with the same culture as was used in the last experiment. The results shown in Table II indicate that concentrations less than 0.1% have very little effect.

TABLE II

Titration of *Cl. welchii* haemotoxin prepared from media containing increasing concentrations of cysteine

Cysteine, %	Toxin concentration											
	$\frac{1}{20}$	$\frac{1}{40}$	$\frac{1}{60}$	$\frac{1}{80}$	$\frac{1}{100}$	$\frac{1}{200}$	$\frac{1}{400}$	$\frac{1}{500}$	$\frac{1}{800}$	$\frac{1}{1000}$	$\frac{1}{2000}$	Cont.
.2	+	–	–	–	–	–	–	–	–	–	–	–
.1	+	–	–	–	–	–	–	–	–	–	–	–
.05	+	+	+	+	+	+	+	+	±	–	–	–
.01	+	+	+	+	+	+	+	+	±	–	–	–
.05	+	+	+	+	+	+	+	+	+	+	±	–
.001	+	+	+	+	+	+	+	+	+	+	±	–

The ability of sulphur in other forms to effect the haemotoxin production of this organism was also examined. Hydrogen sulphide was bubbled through sterile distilled water to the saturation point. The solution was then added to the medium to a concentration of 2%. The medium was sufficiently buffered that this addition of hydrogen sulphide did not alter the pH. As indicated in Table III, *Cl. welchii*, though growing luxuriantly in this medium,

TABLE III

Titration of *Cl. welchii* haemotoxin prepared from media with and without added hydrogen sulphide

—	Toxin concentration									
	$\frac{1}{20}$	$\frac{1}{40}$	$\frac{1}{60}$	$\frac{1}{80}$	$\frac{1}{100}$	$\frac{1}{200}$	$\frac{1}{400}$	$\frac{1}{500}$	$\frac{1}{800}$	Control
Hydrogen sulphide	–	–	–	–	–	–	–	–	–	–
Control	+	+	+	+	+	+	+	+	–	–

failed to produce sufficient haemotoxin to show a reaction in a dilution of 1 in 20 while control toxin prepared in the same batch of medium lacking the hydrogen sulphide gave haemolysis in a dilution of 1 in 500.

In order to ascertain whether the sulphur compounds influenced the metabolism of the organisms or reacted with the haemotoxin, the following experiment was arranged. A sample of toxin prepared in the usual way was divided into two lots. To one lot saturated hydrogen sulphide solution was added to make 2% of saturation as used in the previous experiment, while the other portion was left untreated to serve as a control. The two samples were then sealed with vaseline and allowed to stand for several days. On titration the two toxins gave the same reaction as shown in Table IV. This appears to indicate quite definitely that sulphur or sulphur compounds affect haemotoxin production by *Cl. welchii* through alteration in the metabolism of the organism rather than by acting on the haemotoxin directly.

TABLE IV

TITRATION OF *Cl. welchii* TOXIN WITHOUT ADDED HYDROGEN SULPHIDE AND AFTER STANDING SEVERAL DAYS IN CONTACT WITH HYDROGEN SULPHIDE

—	Toxin concentration										
	$\frac{1}{20}$	$\frac{1}{40}$	$\frac{1}{60}$	$\frac{1}{80}$	$\frac{1}{100}$	$\frac{1}{200}$	$\frac{1}{400}$	$\frac{1}{600}$	$\frac{1}{800}$	$\frac{1}{1000}$	Cont.
H_2S-treated toxin	+	+	+	+	+	+	+	+	±	–	–
Normal toxin, no H_2S	+	+	+	+	+	+	+	+	+	–	–

Oxidation-reduction Potential

It was anticipated that the metabolic influence might result from alteration in the oxidation-reduction potential produced especially by the cysteine. Several determinations have been made by the conventional methods. The particular arrangement of the apparatus was as described earlier by Boyd and Reed (1). The culture vessels consisted of 10 × 1 in. Pyrex test tubes containing 35-cc. amounts of the Robertson's meat medium with or without cysteine. The reaction in each case was adjusted to pH 7.2. These were autoclaved with bright platinum electrodes in place. As soon as sufficiently cooled, potassium chloride-agar bridges were inserted and the sterile tubes sealed with a thick layer of vaseline. These were held for 24 hr. at 37° C. and the initial *sterile* potential reading taken at the end of this period (1). Inoculations were made through the vaseline seal with a fine Pasteur pipette from a young culture in the same medium, the cultures maintained at 37° C., and potential readings made at short intervals for 18 to 24 hr. The results have been calculated in terms of the H electrode as zero.

A characteristic result is shown in the curves (Fig. 1) drawn from the results of an experiment in which three concentrations of cysteine, 0.05, 0.1 and 0.2% were used, together with a control without added cysteine and with a sterile control. The cysteine and control tubes were filled from one flask of Robertson's chopped meat in such a way that each received the same amount of both the solid meat and the fluid portion of the medium. It is apparent that the addition of cysteine up to 0.1% produced no appreciable change and 0.2% produced a barely significant change in potential in the

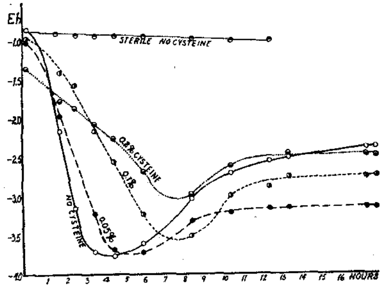

FIG. 1. *Curves indicating change in the oxidation-reduction potential of cultures of Cl. welchii in Robertson's meat media containing various concentrations of cysteine. Ordinates, Eh; abscissa, time in hours.*

sterile medium. This condition doubtless results from an efficient poising effect of some constituent of the medium. On the other hand the poising action of the cysteine is very apparent, particularly during the first six to eight hours' growth of the culture. The curves (Fig. 1) indicate that in the cultures without cysteine there is a precipitous drop in oxidation-reduction potential which begins in slightly less than an hour after inoculation, followed by a gradual rise to an approximate equilibrium at 13–15 hr. Cultures containing 0.05% cysteine show a similar, although slightly more delayed, drop, followed by a more gradual rise. The 0.1 and the 0.2% cysteine cause a much greater delay in the initial fall and definitely restrict the extent of the fall. This appears unrelated to the rate of growth of the organisms. Although no quantitative determinations were made, the rate of pH change in the controls and in the cysteine-containing media was approximately the same and the first evolution of gas occurred in all the tubes at two and a half to three hours after inoculation. Since the pH change was approximately the same in all the tubes, from 7.2 to 6.2 six to eight hours after inoculation, no correction for this change has been made in the curves. Part, but not all, of the positive drift six to eight hours after the inoculation is the result of the pH change.

It appears not unreasonable to conclude that the marked differences in the oxidation-reduction of the medium during the most active period of the growth of the organisms may have a profound influence on the metabolism, and result in the observed differences in haemotoxin production.

Cystine Content of Various Media

Sullivan and Hess (8), employing several different methods for the quantitative estimation of cystine have demonstrated the existence of a very appreciable difference in the amount of this amino acid contained in various kinds of meat and fish. As shown in Table V, while there is some slight variation in the amount of cystine shown in the materials tested by the different methods, all are in agreement so far as showing that fish muscle contains in the neighborhood of twice the amount of cystine contained in beef muscle.

A series of media were made up similar to Robertson's chopped meat, using sirloin and round beef steak, salmon and halibut muscle. The media were

TABLE V

CYSTINE CONTENT OF FRESH MEAT AND FISH

—	Cystine on moist weight, %				Cystine on dry weight, %			
	Sullivan method	Okuda method	Folin Marenzi method	Average	Sullivan method	Okuda method	Folin Marenzi method	Average
Round steak	.132	.130	.174	.145	.522	.516	.693	.577
Sirloin steak	.118	.117	.194	.143	.194	.480	.474	.382
Haddock	.231	.231	.387	.283	1.16	1.16	1.94	1.42
Halibut	.238	.231	.408	.292	1.13	1.09	1.94	1.38
Salmon	.236	.243	.418	.299	.85	.88	1.50	1.07

TABLE VI

TITRATION OF *Cl. welchii* TOXIN PREPARED FROM MEAT AND FISH MEDIA

—	Toxin concentration										
	$\frac{1}{20}$	$\frac{1}{40}$	$\frac{1}{60}$	$\frac{1}{80}$	$\frac{1}{100}$	$\frac{1}{200}$	$\frac{1}{400}$	$\frac{1}{600}$	$\frac{1}{800}$	$\frac{1}{1000}$	Cont.
Sirloin steak	+	+	+	+	+	+	±	±	–	–	–
Round steak	+	+	+	+	+	+	±	–	–	–	–
Halibut	+	+	+	±	±	–	–	–	–	–	–
Salmon	+	+	+	±	±	–	–	–	–	–	–

all prepared in the same manner, all adjusted to pH 7.2 and dispensed to small flasks with care that all the flasks received the same proportion of solid and fluid portions of the media. The percentage content of cystine to be expected in the several media from Sullivan and Hess' results is shown in the last column of Table II. It is not apparent, however, what proportion is present as free cysteine or combined in proteins. The media were all inoculated with the same toxin producing strain of *Cl. welchii* and filtrates recovered from all after 22 hours' incubation. The results of haemotoxin titration are shown in Table III. It is apparent that the beef muscle media with a low cysteine content give a high yield of toxin and the fish muscle media with a much higher content of cystine give a very much lower yield. It is of course probable that other constituents of the two media are also concerned.

Conclusion

1. It has been shown that the addition of 0.1% or more of cysteine to Robertson's chopped meat media completely inhibits the production of haemotoxin by *Cl. welchii*, and that hydrogen sulphide has a similar effect.

2. The effect is shown to be related to the metabolism of the organisms and not to a direct reaction with the formed toxin.

3. The addition of these concentrations of cysteine were shown to have little effect upon the oxidation-reduction potential of sterile media but a marked effect upon the oxidation-reduction potential of cultures during the most active growth period.

4. Beef muscle media low in cystine was shown to give a good yield, and fish muscle relatively high in cystine was shown to give a poor yield of haemotoxin.

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DERIVATIVES OF SUBSTITUTED SUCCINIC ACIDS

I. THE ACTION OF ALKALINE SODIUM HYPOBROMITE ON 1,2-DIPHENYL-SUCCINAMIDE AND 3,4-DIPHENYLSUCCINIMIDE[1]

By John A. McRae[2] and Alvin S. Townshend[3]

Abstract

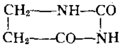
The application of the Hofmann reaction for the preparation of amines from amides to 1:2-diphenylsuccinamide has led to none of the expected substances, but to diphenylacetic acid as the principal product of the reaction. The isolation of this substance indicates that at some stage a rearrangement has occurred. The mechanism of the rearrangement is obscure but oxidation to a 1,2-dihydroxy-diamide may be the first stage. This is supported by the conversion of diphenyl-succinimide into diphenylmaleic anhydride by alkaline sodium hypobromite.

Various derivatives of diphenylsuccinic acid, which were obtained in unsuccessful attempts to prepare in quantity other 1:2-diarylsuccinamides, are described.

Introduction

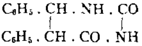
The preparation of substituted succinic acids through addition of hydrogen cyanide to substances of the types RCH : C(CN)CO_2H and RCH : C(C_6H_5)CN was studied some years ago by Lapworth and McRae (8). It has seemed desirable to study in several directions the behavior of the acids thus obtained and that of related compounds.

Through earlier work (9) on the action of alkaline sodium hypobromite on phenyl- and piperonyl- succinimides, in which it was found that hydrolysis to the corresponding succinic acids occurred exclusively, the writers' attention was turned first of all to the action of this reagent on 1,2-diphenylsuccinamide which had been obtained by the hydrolysis of 1,2-diphenylsuccinonitrile (8). The only amides of the succinic acid series whose behavior towards sodium hypobromite has been studied previously are succinamide and methyl-succinamide. Weidel and Roithner (11) observed that these amides behaved similarly to phthalamide (7) giving the cyclic ureides, dihydrouracil (I) and methyldihydrouracil, respectively.



(I)

Accordingly it was expected that 1,2- diphenylsuccinamide would yield diphenyldihydrouracil (II), or alternatively 1,2-diphenylethylene diamine, αβ-diphenyl-β-aminopropionic acid or some other of the various types of substances that sometime arise in the application of the Hofmann reaction.



(II)

None of the expected substances was found when 1,2-diphenylsuccinamide (presumably the meso- form) was subjected to the action of alkaline sodium hypobromite as employed in the Hofmann reaction. Cold alkaline sodium hypobromite slowly dissolves this amide and when the solution is heated to 75–80° C., after first adding the appropriate quantity of sodium hydroxide,

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a small amount of alkali-insoluble material is produced, identified as principally diphenylsuccinamide; benzaldehyde and ammonia are formed to a certain extent and on acidifying the filtrate, diphenylacetic acid is precipitated as the principal product of the reaction. It is accompanied by traces of other substances from which it is freed with some difficulty. The yield of diphenylacetic acid varies considerably but in some experiments it has been as high as 90%. Similar results have been obtained using alkaline sodium hypochlorite.

The conversion of diphenylsuccinamide into diphenylacetic acid adds another example to the already numerous instances of rearrangements which various derivatives of 1,2-diphenylethane undergo, although as far as the writers have been able to ascertain alkaline sodium hypobromite has not been found previously to cause such changes. The rearrangement observed is not to be ascribed to the action of hot caustic soda on the diamide, for at the concentration of alkali and at the temperature employed in this attempted Hofmann reaction, caustic soda is without appreciable effect on diphenylsuccinamide. In the second place, although a residue of this amide remains, yet the original diphenylsuccinamide is dissolved by cold sodium hypobromite solution. The authors hope to report shortly on the nature of the changes at this stage. In view of the fact that Feist and Arnstein (5) observed that 1,2-diphenylethylene diamine, when treated with nitrous acid, gave hydrobenzoin and diphenylacetaldehyde, the possibility that this diamine had been formed during the reaction and then either had been oxidized to hydrobenzoin or had undergone rearrangement was investigated. Diphenylethylene diamine was prepared according to Feist (4) by reduction of α-benzildioxime. In this reduction not only was there isolated the racemic form (m.p. 90–91° C.) of the diamine that Feist had obtained, but also the meso- form (m.p. 120° C.) obtained by Grossmann (6) from amarin. Using the same conditions of alkalinity and temperature as were used with diphenylsuccinamide both of these forms with alkaline sodium hypobromite gave benzaldehyde and ammonia, but we were unable to isolate any diphenylacetic acid.

Another possibility is that the hypobromite oxidizes diphenylsuccinamide to $\alpha\alpha'$-dihydroxy- $\alpha\alpha'$-diphenylsuccinamide (III) and the observed changes arise from the further transformations of this substance. Evidence will be presented in a forthcoming paper to support this view, but the probability of such an attack

$$\begin{array}{c} C_6H_5\,.\,C(OH)\,.\,CONH_2 \\ | \\ C_6H_5\,.\,C(OH)\,.\,CONH_2 \end{array}$$

(III)

is strengthened by the fact that when 3,4-diphenylsuccinimide was subjected to the action of sodium hypobromite, under the conditions of the Hofmann reaction used for converting phthalimide into anthranilic acid, the expected $\alpha\beta$-diphenyl-β-aminopropionic acid was not isolated, but instead diphenylsuccinamic acid, $C_6H_5CH\,.\,(CONH_2)\,CH(C_6H_5)CO_2H$, and diphenylmaleic anhydride were obtained. The latter was formed most probably by loss of water from α-hydroxy- $\alpha\alpha'$-diphenylsuccinamide, formed by oxidation and hydrolysis of the imide. When diphenylsuccinamic acid was treated with the alkaline hypobromite solution a mixture of acidic substances was produced from which only benzoic acid was isolated.

Attempts were made to ascertain whether the arrangement observed with diphenylsuccinamide and sodium hypobromite was characteristic of 1,2-diarylsuccinamides. For this purpose *pp'*-dinitrodiphenylsuccinamide was prepared by the nitration of diphenylsuccinamide. Its orientation follows from its hydrolysis to the corresponding acid identical with the *pp'*-dinitrodiphenylsuccinic acid described by Reimer (10). Unsuccessful attempts were made to prepare in quantity *p*-methoxydiphenylsuccinamide and *o*-chlorodiphenylsuccinamide. As has also been shown by Brand and Loehr (2) anisylidenebenzyl cyanide readily unites with hydrogen cyanide but the resulting dinitrile, $CH_3OC_6H_4CH(CN)CH(C_6H_5)CN$, could not be hydrolyzed to the diamide. Using a concentration of sulphuric acid appropriate for the hydrolysis, sulphonation occurred. Further heating of the sulphonated product with moderately concentrated sulphuric acid gave *p*-methoxydiphenylsuccinic acid which was prepared by Brand and Loehr by direct hydrolysis. *o*-Chlorophenylcinnamonitrile with hydrogen cyanide unexpectedly gave a tarry product from which the writers were unable to isolate any of the expected dinitrile, but hydrolysis of the material yielded small amounts of *o*-chlorodiphenylsuccinamide and *o*-chlorodiphenylsuccinic acid.

The procedure used with diphenylsuccinamide was applied to its dinitro-derivative, but the only substance isolated in amount sufficient for identification was the half-amide of *pp'*-dinitrodiphenylsuccinic acid.

Later work (3) however has shown that under the influence of alkaline sodium hypobromite, 1,2-*p*-tolylphenylsuccinamide gives *p*-tolylphenylacetic acid, and 1,2-*p*-chlorodiphenylsuccinamide gives *p*-chlorodiphenylacetic acid. It may, therefore, be considered that the observed rearrangement is general with 1,2-diarylsuccinamides.

Experimental

Action of Sodium Hypobromite on Diphenylsuccinamide

s-Diphenylsuccinamide was made according to the directions of Lapworth and McRae (8). In general the product obtained from the hydrolysis of diphenylsuccinonitrile was used directly after a thorough digestion with cold sodium hydroxide solution. Using a recrystallized sample of the amide, the same results were obtained as with material which had not been recrystallized.

The hypobromite solution was made by dropping slowly, with efficient stirring, 32 gm. of bromine into a solution of 40 gm. of sodium hydroxide in 200 cc. of water cooled to −10° C. The diphenylsuccinamide (26.8 gm.), made into a thin paste with a little water, was added slowly, the temperature being maintained below 0° C. The agitation was continued for one-half hour. All but a very small residue dissolved. When the temperature had risen to 0° C., 24 gm. of sodium hydroxide was added with stirring and the mixture heated on the water bath to 75–80° C. for three hours. During the heating some ammonia and benzaldehyde were formed and a further precipitate settled out. After dilution this was collected and recrystallized from glacial acetic acid; m.p. 310° C. It did not depress the melting point of diphenylsuccinamide.

Analysis confirmed the identity of the substance as diphenylsuccinamide. Calcd. for $C_{16}H_{16}O_2N_2$: N, 10.45%. Found: N, 10.90%. The substance was identified further by hydrolysis to diphenylsuccinic acid; m.p. 229° C. It is accompanied apparently at times by a small amount of a second substance, comparatively readily soluble in alcohol. On recrystallization from alcohol it melted at 308° C. and depressed the melting point of the substance with which it was associated to 254° C.

The filtrate was extracted with ether but only traces of bases or other ether-soluble substances were obtained. On acidification with hydrochloric acid a yellowish semi-oily precipitate was formed from which carbon dioxide seemed to be evolved gradually. When the precipitate had hardened it was collected, dissolved in sodium hydroxide and the alkaline solution boiled with charcoal. On acidification fairly pure diphenylacetic acid was obtained; yield, 17 gm. It was recrystallized several times from hot water; m.p. 146° C. The equivalent calculated for $C_{14}H_{12}O_2$ is 212; found, 212.5. The substance was compared, and found to be identical, with a sample of diphenylacetic acid, purchased from British Drug Houses, which on recrystallization melted at 146° C. Comparison was made also with a specimen of the acid made by reducing benzilic acid.

A portion of the diphenylacetic acid thus obtained from diphenylsuccinamide was oxidized with chromic acid in glacial acetic acid to benzophenone (m.p. 46° C.). Other portions were converted into the ethyl ester (m.p. 58° C.) and into the anilide (m.p. 180° C.). In each case the preparations were compared with the same derivatives made from authentic diphenylacetic acid, and identified further by analysis.

In an experiment in which the reaction mixture was heated for but one-half hour at 80° C. the yield of diphenylacetic acid fell to 50%. Using one molecular proportion of bromine, instead of two, to one of amide nearly all of the amide was recovered unchanged. Prolonged heating of diphenylsuccinamide on the water bath with a solution of sodium hydroxide, of the same concentration as in the experiments with sodium hypobromite, caused no change in the amide.

Action of Alkaline Sodium Hypobromite on 1,2-Diphenylethylenediamine

α-Benzildioxime (10 gm.) was reduced with sodium and absolute alcohol according to the directions given by Feist (4) and Feist and Arnstein (5). When reduction was complete, the solution was diluted with water and acidified with hydrochloric acid. The alcohol was then distilled off. In addition to the sodium chloride which separated during the distillation, the residual liquid on cooling deposited 2.4 gm. of a crystalline solid. The filtrate on treatment in the usual way gave Feist's diphenylethylenediamine (m.p. 90–92° C.). On redissolving the 2.4 gm. of material in water and then making the solution alkaline, a base precipitated. On recrystallization it melted at 120° C. Its properties agree with those of the diphenylethylenediamine obtained by Grossmann (6) from amarin. Calcd. for $C_{14}H_{16}N_2$: N, 13.2%. Found: N, 13.3%.

By substituting each of these diphenylethylenediamines for diphenylsuccinamide, under the foregoing conditions with sodium hypobromite, ammonia and benzaldehyde were formed on warming. A gummy mass formed and on removal of this nothing further separated on acidification.

α-Phenyl-o-chlorocinnamonitrile, $ClC_6H_4CH : C(C_6H_5)CN$

This substance was made by condensing equimolecular proportions of *o*-chlorobenzaldehyde and benzyl cyanide, dissolved in twice their weight of alcohol, by means of a few drops of 50% caustic soda. The condensation product which is formed quickly was obtained in colorless needles after several recrystallizations from alcohol; m.p. 107° C. Calcd. for $C_{15}H_{10}NCl$: C, 75.12; H, 4.17; N, 5.84%. Found: C, 75.55; H, 4.40; N, 6.10%.

Attempted Preparation of o-Chlorodiphenylsuccinonitrile

The procedure was that which has been used here repeatedly for similarly constituted nitriles. *α*-Phenyl-*o*-chlorocinnamonitrile (24 gm.) was dissolved in 700 cc. of alcohol at 50–60° C. A solution of 27.4 gm. of potassium cyanide in 80 cc. of water was added and the temperature maintained in this range for an hour. A solution of acetic acid (12 gm. in 50 cc. water) was then introduced gradually at the bottom of the solution. The mixture was allowed to stand for three days. It became very dark brown but nothing separated. About 600 cc. of alcohol was removed by distillation and the residue diluted copiously with water. A large quantity of a brown spongy mass separated and from this it was not possible to obtain a pure substance by crystallization.

A portion (10 gm.) of this crude product was dissolved in warm 80% sulphuric acid and allowed to stand overnight. On diluting with water a precipitate formed. After it was digested with caustic soda, 2.5 gm. of insoluble material was left. This was recrystallized from a large volume of hot acetic acid and seemed to be *o*-chlorodiphenylsuccinamide. The recrystallized material softened at 278° and melted at 280° C. Calcd. for $C_{16}H_{15}O_2N_2Cl$: C, 63.5; H, 4.95; N, 9.25%. Found: C, 63.5; H, 4.85; N, 9.75%. The caustic soda solution gave a mixture of acids from which no single substance was isolated.

A second portion of 10 gm. of the crude product was dissolved in 90% sulphuric acid and, after dilution until the acid had a strength of about 70%, was heated at 120° C. for four hours. Further dilution gave a mixture of acidic substances from which, by recrystallization from alcohol, an acid, m.p. 217° C., was isolated ultimately in small yield. The analytical figures agree with those for *o*-chlorodiphenylsuccinic acid. Calcd. for $C_{16}H_{13}O_4Cl$: C, 63.1; H, 4.27%; equivalent, 152.3. Found, C, 63.1; H, 4.32%; equivalent 153.8.

Some later experiments performed with the assistance of Mr. J. G. Craig showed that *α*-phenyl-*o*-chlorocinnamonitrile reacts with hydrogen cyanide much faster than does *α*-phenylcinnamonitrile, and an attempt was made to isolate the addition compound by using milder conditions.

A hot solution of 9.6 gm. of α-phenyl-*o*-chlorocinnamonitrile in 100 cc. of alcohol was mixed with 5.2 gm. of potassium cyanide in 15 cc. of water and kept gently simmering for two hours. The mixture gradually acquired a deep brown color. On cooling and diluting with water a brown, amorphous precipitate (about 1 gm.) separated. The filtrate on acidification deposited 8 gm. of a crystalline precipitate. After several recrystallizations from hot benzene or hot dilute alcohol the substance was obtained in rhomboidal plates, m.p. 135° C. Although the melting point seems low, the analytical figures indicate that the substance is the half nitrile of *o*-chlorodiphenylsuccinic acid (presumably $C_6H_5 . CH(CN)C_6H_4Cl . CO_2H$, since diphenylsuccinonitrile is stable under similar conditions). Calcd. for $C_{16}H_{12}O_2NCl$: N, 4.95; Cl, 12.44%. Found: N, 4.95; Cl, 12.65%.

pp'-Dinitrodiphenylsuccinamide,

$$CONH_2 . CH(C_6H_4NO_2) . CH(C_6H_4NO_2) . CONH_2$$

Diphenylsuccinic nitrile (25 gm.) was dissolved in 275 cc. of 90% sulphuric acid on the water bath, cooled to room temperature and nitrated with efficient stirring with excess nitric acid. After standing four hours the mixture was poured on ice and the precipitated amide was washed with sodium carbonate. The yield at this stage was 30.2 gm. (80% of theoretical). The substance is very difficultly soluble in the usual organic solvents but was recrystallized by dissolving it in hot phenol and, after the solution had cooled, precipitating with acetone. The pure substance is faintly yellow, and melts at 294° C. with decomposition. Calcd. for $C_{16}H_{14}O_6N_4$: C, 53.6; H, 4.91; N, 15.64%. Found: C, 53.2; H, 3.28; N, 15.64. It gave, on hydrolysis with 90% sulphuric acid at 120° C., dinitrodiphenylsuccinic acid, m.p. 242° C., which was seemingly identical in properties with the acid described by Reimer (10). It was oxidized to *p*-nitrobenzoic acid by chromic acid dissolved in glacial acetic acid.

The action of alkaline sodium hypobromite on this amide, using the same conditions as with diphenylsuccinamide, brought about the evolution of ammonia as the amide dissolved. Acidification gave an amorphous precipitate from which a very small proportion of *pp'*-dinitrodiphenylsuccinamic acid was obtained. This substance after recrystallization from alcohol softened at 230° C. and gradually decomposed as the temperature was raised. Calcd. for $C_{16}H_{13}O_7N_3$: N, 14.6%; equivalent 359. Found: N, 15.0%; equivalent 359.

p-Methoxydiphenylsuccinonitrile

This substance was prepared by the method used by Lapworth and McRae (8) for diphenylsuccinonitrile. Later the slightly different method of Brand and Loehr was used with equally good results. Recrystallized from glacial acetic acid, the substance was obtained in fine, colorless needles; m.p. 204° C. Brand and Loehr give the melting point as 193° C. Calcd for $C_{17}H_{14}ON_2$: C, 77.9; H, 5.35; N, 10.68%. Found: C, 77.5; H, 5.36; N, 11.2%.

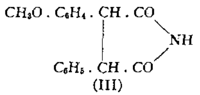
All attempts to hydrolyze this dinitrile to the corresponding diamide failed. Using sulphuric acid of various strengths, fuming hydrochloric acid or alcoholic

potash, the substance was in general either not attacked or else hydrolyzed to *p*-methoxydiphenylsuccinic acid. In some attempts with concentrated sulphuric acid sulphonation occurred, as observed by Brand and Loehr. These authors obtained *p*-methoxydiphenylsuccinic acid as needles from xylene; m.p. 221° C., whereas the writers' specimen of this acid, recrystallized from alcohol or acetic acid, melted at 227° C. Calcd. for $C_{17}H_{16}O_5$: C, 68.0; H, 5.3%. Found: C, 67.9; H, 5.1%.

Diethyl p-Methoxydiphenylsuccinate, $CH_3O\,.\,C_6H_4\,.\,CH(CO_2C_2H_5)\,.\,(C_6H_5)CH\,.\,CO_2C_2H_5$. This was obtained by esterifying the foregoing acid with ethyl alcohol and sulphuric acid. Recrystallized from alcohol it melted at 102° C. Calcd. for $C_{21}H_{24}O_5$: C, 70.8; H, 6.7%. Found: C, 70.9; H, 6.8%. It was accompanied by the half ester, ethyl *p*-methoxydiphenylsuccinate, $CH_3OC_6H_4(CO_2Et)C_6H_5CHCO_2H$ (?) from which it was separated by means of sodium carbonate. The latter substance was recrystallized from alcohol; m.p. 154° C. Calcd. for $C_{19}H_{20}O_5$: C, 69.5; H, 6.1%; equivalent, 328. Found: C, 69.4; H, 5.8%; equivalent, 329.

p-Methoxydiphenylsuccinimide (III)

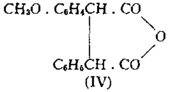
Ammonium *p*-methoxydiphenylsuccinate (10 gm.) was heated in a flask at 12 mm. until the residue began to char. The red gummy distillate was extracted with acetone, and from the acetone extract 6.1 gm. of crude imide was obtained. This on recrystallization from alcohol gave colorless minute needles, m.p. 178° C. It may also be recrystallized from benzene. Calcd. for $C_{17}H_{15}O_3N$: C, 72.2; H, 5.3; N, 5.0%. Found: C, 72.0; H, 5.5; N, 5.5%.



(III)

p-Methoxydiphenylsuccinic Anhydride (IV)

An attempt to prepare this substance by distilling *p*-methoxydiphenylsuccinic acid at 12 mm. gave a thick gummy distillate (soluble in ether) from which the desired anhydride could not be isolated.



(IV)

It was obtained easily by dissolving 10 gm. of the acid in 15 cc. of thionyl chloride with gentle warming. The excess thionyl chloride was distilled off and the residue recrystallized from benzene by the addition of petroleum ether. Star-shaped crystals melting at 114° C. were obtained. Calcd. for $C_{17}H_{14}O_3$: C, 72.3; H, 5.0%. Found: C, 72.8; H, 5.1%.

Action of Sodium Hypobromite on s-Diphenylsuccinimide

Diphenylsuccinimide was made from ammonium diphenylsuccinate according to the directions of Lapworth and McRae (8), but it was found that it could be made more readily by heating diphenylsuccinamide in a Claisen flask at 15 mm. pressure until the evolution of ammonia ceased. The distillate and the residue in the flask were thoroughly extracted with benzene and the diphenylsuccinimide thus obtained recrystallized from benzene. Twenty grams of amide gave 13 gm. of imide.

Diphenylsuccinimide (18.6 gm.), made into a thin paste with a little water, was stirred into a cold solution of sodium hypobromite prepared by dissolving 12 gm. of bromine in a solution of 15 gm. of sodium hydroxide in 75 cc. of water at $-10°$ C. The imide did not appear to dissolve but formed a pasty mass. When the temperature had risen to 0° C., 9 gm. of sodium hydroxide was added and the mixture heated for three hours on the water bath at 80° C. The imide dissolved and ammonia was evolved. The solution, after cooling, was acidified with hydrochloric acid. The crude product obtained consisted chiefly of diphenylsuccinamic acid and diphenylmaleic anhydride. These were separated by heating with chloroform in which the former substance is insoluble. From 16 gm. of crude product, 9.3 gm. of the amido-acid and 6.2 gm. of diphenylmaleic anhydride were obtained.

Diphenylsuccinamic acid was recrystallized from ethyl alcohol. It softens at 215° C. and melts with decomposition at 218° C. Calcd. for $C_{16}H_{15}O_3N$: C, 71.4; H, 5.57; N, 5.20%; equivalent 269. Found: C, 70.6; H, 5.7; N, 5.36%; equivalent 270.5. Hydrolysis with 90% sulphuric acid gave diphenylsuccinic acid.

Diphenylmaleic anhydride was recrystallized from dilute alcohol in needles; m.p. 155° C. Calcd. for $C_{16}H_{12}O_2$: C, 76.8; H, 4.0%. Found: C, 76.2; H, 4.1%. The substance obtained agrees in properties with the description given by Reimer (10). Potassium permanganate oxidized it to benzoic acid, and chromic acid in acetic acid oxidized it to benzil. It reduced ammoniacal silver nitrate and was converted by ammonia into diphenylmaleic imide; m.p. 214° C. Calcd. for $C_{16}H_{11}O_2N$: C, 77.1; H, 4.4; N, 5.6%. Found: C, 77.3; H, 4.5; N, 5.9%.

Under the conditions used by Hoogewerff and van Dorp (7) in their experiments on succinimide, somewhat different results were obtained. Diphenylsuccinimide (12.5 gm.) was added to a cold hypobromite solution made from 8 gm. of bromine added to 16 gm. of sodium hydroxide in 150 cc. of water and then heated on the water bath. Ammonia was evolved and the product obtained on acidification (10.7 gm.) gave 5.2 gm. of diphenylmaleic anhydride when treated as before. No amido-acid could be isolated from the part insoluble in chloroform.

Action of Sodium Hypobromite on Diphenylsuccinamic Acid

Using the same conditions as with the amide, diphenylsuccinamic acid was treated with alkaline sodium hypobromite solution. The only substance that could be identified after acidification was benzoic acid.

1,2-Diphenylsuccinyl Chloride (V)

Diphenylsuccinic acid (12 gm.) was mixed intimately in a flask with 22.1 gm. of phosphorus pentachloride. Reaction occurred slowly and the mixture was warmed gently on the water bath until it had become quite gummy. Phosphorus oxychloride and unchanged phosphorus pentachloride were removed by heating on the water bath under reduced pressure. Attempts to distil the acid chloride

$$\begin{array}{l} C_6H_5CH.COCl \\ \quad | \\ C_6H_5CH.COCl \end{array}$$

(V)

under reduced pressure were unsuccessful. The residue was dissolved in hot benzene and recrystallized from the same solvent, from which the acid chloride was deposited as flaky colorless crystals; m.p. 190° C. It was not readily attacked by water but it was rapidly dissolved by hot alkali from which acids precipitated diphenylsuccinic acid. Calcd. for $C_{16}H_{12}O_2Cl_2$: C, 62.5; H, 3.91; Cl, 23.1%. Found: C, 62.6; H, 3.9; Cl, 23.4%. It was converted by grinding with ammonium carbonate into diphenylsuccinamide. On warming with ethyl alcohol the diethyl ester of mesodiphenylsuccinic acid, m.p. 139° C., was obtained. Anschütz and Bendix (1) give the melting point as 140–141° C. The acid chloride is therefore the symmetrical dichloride of β- or mesodiphenylsuccinic acid.

In some preparations the acid chloride was accompanied by a greenish-yellow substance which was separated by dissolving it in ether. The substance was identified as diphenylmaleimide and evidently arose from the presence of diphenylsuccinamic acid in the diphenylsuccinic acid used. By digesting diphenylsuccinamide (3 gm.) with phosphorus pentachloride it was not found possible to isolate diphenylmaleimide, but by warming the crude product with dilute caustic soda and acidifying, 2 gm. of diphenylmaleic anhydride was obtained.

Acknowledgments

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THE PREPARATION AND PHYSICAL PROPERTIES OF ALIPHATIC ACETYLENES[1]

By F. R. Morehouse[2] and O. Maass[3]

Abstract

This paper deals with the preparation and determination of the physical properties of the lower members of the acetylene series—methyl, ethyl, dimethyl and propyl acetylenes. A comprehensive determination of the physical properties of these compounds has been made and the results have been interpreted along with similar data obtained for the paraffin and olefine series. The acetylenes have been found to differ from the paraffins and olefines. The difference is attributed to the polarity of the acetylenes as a group. This is further substantiated by a comparison of the properties of the two isomeric acetylenes, dimethyl and ethyl acetylenes.

Introduction

A systematic study of the physical properties of the paraffin and the olefine hydrocarbons has been the subject of investigation in this laboratory. Those hydrocarbons containing two and three carbon atoms were investigated by Wright and Maass (10), and the properties of the four-carbon paraffins and olefines were determined by Coffin and Maass (3). The physical constants of the first four-carbon acetylene have been given in a previous publication accompanied by a redetermination of the properties of methyl acetylene (11). In this paper the physical constants of dimethyl acetylene will be added to those already determined for the other acetylenes and a comparison made, on the basis of these physical properties, of the paraffin, olefine and acetylene series from the point of view of their molecular structure.

Experimental

In order to obtain the acetylenes in the highest state of purity they were prepared by direct synthesis from the simple constituents of each compound. This method, with certain modifications, was that used originally by Lebeau and Picon (8) and consists in the alkylation of sodium acetylide in liquid ammonia as a reaction medium. Since this method and modifications have been previously described in detail (11) they will not be repeated. The preparation of propyl acetylene was carried out in the usual manner. Dimethyl acetylene was prepared by the methylation of the sodium derivative of methyl acetylene in a manner analogous to that used in the preparation of the other alkyl acetylenes. A large quantity of methyl acetylene was prepared and passed into the blue solution of sodium in liquid ammonia. Disappearance of the blue color showed complete formation of sodium methyl acetylide. Lebeau (9) found this reaction to take place in the following way:

$$3CH_3C\equiv CH + 2Na = 2CH_3C\equiv CNa + CH_3CH=CH_2$$

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[2] *Holder of a studentship under the National Research Council of Canada at the time during which part of the investigation was carried out.*
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When all the sodium had reacted with the methyl acetylene, the ammonia and propylene were allowed to pass off, leaving the white, solid sodium derivative behind. As the mercury and silver derivatives of methyl acetylene are non-explosive (6, 7) this procedure was considered safe. Methylation was then carried out and the dimethyl acetylene separated in the usual manner. As there were discrepancies in the results for the parachor, compared with those for the other acetylenes, it was thought advisable to prove the identity of the dimethyl acetylene obtained in this way.

By determining the vapor density of the product the molecular weight was found to agree with the theoretical value for dimethyl acetylene within 1%. As the boiling point of the product was found to be 27.1° C., which agrees with the values found by other investigators (1), 27.2° and 27.6° C., it was not considered likely that the product could be one of the isomers, 1, 2-butadiene and 1, 3-butadiene, since the boiling points are given in the International Critical Tables as 19.0° C. for the former and −2.6° C. for the latter. The boiling point of the isomeric ethyl acetylene has been found to be 8.6° C. As a final check, however, the compound was brominated and analyzed. The melting point of the brominated compound, 210° C., agrees reasonably with the value determined by Faworsky (4) and is quite different from the melting points of the bromination products of possible isomers (2). The results of analysis gave 86.6% bromine, as compared to the theoretical 85.5%.

Final purification of all compounds, after they had been separated from ammonia, was effected by fractional distillation (3). The final, pure fractions were then distilled into the apparatus used for the determination of the different physical properties, such as density, melting point, surface tension, etc.

Physical Properties of the Hydrocarbons

The temperature baths used in the determination of the densities, surface tensions, vapor pressures and melting points consisted of a liquid, suitable for the temperature range under which the observations were being carried out, contained in a transparent Dewar flask. All baths were kept vigorously stirred with a current of dry air bubbles, and temperatures were measured with a standard thermometer to 0.1° C. Solid carbon dioxide was used as the cooling agent from 0 to −78° C. and the bath over this temperature range consisted of acetone or ether. For the measurement of properties at lower temperatures high test gasoline, cooled by means of liquid air dropped into a Pyrex test tube immersed in the bath, was used.

The data for methyl and ethyl acetylenes have already been given (11). In Figs. 1, 2 and 3, however, are shown, for purposes of comparison, a graphical representation of the various properties of methyl, ethyl, propyl and dimethyl acetylenes.

Density

Density determinations were made by the dilatometer method described by Wright and Maass (10). The dilatometer was filled by distilling the pure

compound into it, thus obviating the possibility of any contamination by impurities. All the pieces of apparatus used in determining the different physical properties were filled in the same way. In Fig. 1 will be found the densities of dimethyl and propyl acetylenes.

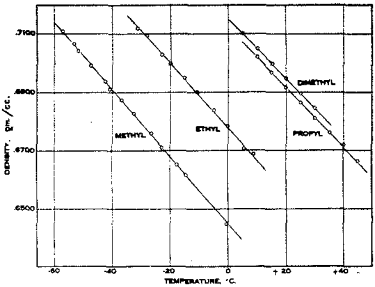

FIG. 1. *Density of methyl, ethyl, propyl and dimethyl acetylenes.*

Surface Tension

The surface tensions of the acetylenes were determined by the capillary rise method. A description of this method and the accuracy obtainable are to be found in the paper referred to above (10). The curves derived for all the surface tensions are to be found in Fig. 2.

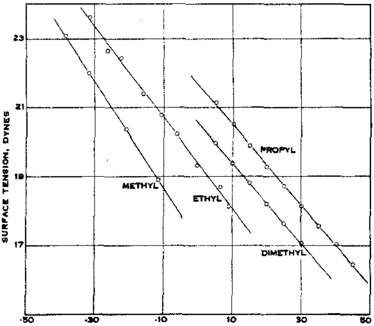

FIG. 2. *Surface tension of methyl, ethyl, propyl and dimethyl acetylenes.*

Melting Points

Melting points were determined by freezing the hydrocarbons in small bulbs and allowing the bath to warm up slowly. The temperature taken as the melting point was halfway between the temperature at which melting began and that at which the solid just disappeared. In all cases a sharp melting point was observed. The melting points are listed with other properties in Table I.

Vapor Pressures

Vapor pressure curves for the hydrocarbons whose boiling points were below room temperature were determined in the fractionating apparatus. For those compounds having boiling points about or above room temperature a small bulb containing the hydrocarbon was sealed to a manometer, and both were immersed in a bath whose temperature could be regulated. This avoided condensation in the connecting tubing and manometer. In Fig. 3 will be found vapor pressure curves of the four hydrocarbons.

TABLE I

PHYSICAL PROPERTIES OF THE FIRST THREE OR FOUR MEMBERS OF THE PARAFFINS, OLEFINES AND ACETYLENES

Compound	M.p., °C.	B.p., °C.	*M.L.H.*	D_b	S_b	Total *S.E.*	*K'* (*R.S.*)	$\frac{M.L.H.}{T_b}$	t_c Calc.	$\frac{T_b}{T_c}$	V_m	Parachor Obs.	Parachor Calc.	Parachor Diff., %
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Ethane	−172.0	−88.3	3880	.5469	16.05	45.7	1.98	21.0	34.6	.60	54.9	110.4	112.2	1.6
Propane	−189.9	−44.5	4700	.5853	15.63	50.3	2.15	20.6	91.6	.62	75.0	150.6	151.2	0.4
n-Butane	−135.0	− 0.5	5597	.6014	14.90	47.8	2.21	20.5	147.0	.64	96.3	190.6	190.2	0.2
i-Butane	−145.0	−10.2	5480	.5944	14.10	47.2	2.23	20.8	129.5	.64	97.6	188.8	190.2	0.2
Ethylene	−169.4	−103.9	3510	.5699	16.50	47.7	2.14	20.8	5.9	.60	49.1	99.4	101.2	1.8
Propylene	−185.2	−47.0	4600	.6095	16.70	49.7	2.12	19.0	91.3	.62	69.0	139.7	140.2	0.4
α-Butylene	−190.0	− 6.1	5400	.6250	15.95	51.1	2.21	20.2	145.0	.64	89.5	179.9	179.2	0.4
β-Butylene	−127.0	1.0	5400	.6300	16.78	51.2	2.14	19.7	156.0	.64	88.9	178.7	179.2	0.3
γ-Butylene	−146.8	− 6.6	5200	.6268	15.72	49.8	2.15	19.7	147.0	.64	89.3	178.4	179.2	0.4
Acetylene	− 81.0	−83.6	4273	.6208	19.58	56.0	2.02	22.2	39.7	.61	41.9	88.1	90.4	2.6
Methyl acetylene	−101.5	−23.3	5562	.6698	20.70	59.4	1.99	22.3	121.6	.62	59.7	127.5	129.4	1.5
Ethyl acetylene	−122.5	8.6	5983	.6682	18.30	53.5	1.96	21.3	190.5	.61	80.8	167.2	168.6	0.8
Dimethyl acetylene	− 24.0	27.1	6434	.6873	17.40	51.8	1.75	21.4	215.5	.61	78.6	160.7	168.6	4.6
Propyl acetylene	− 98.0	39.7	7087	.6718	17.00	53.3	2.12	22.6	220.3	.63	101.3	206.2	207.2	0.5

NOTE:—*M.L.H.*, *molecular latent heat of evaporation;* D_b, *density at boiling point;* S_b, *surface tension at the boiling point; S.E., surface energy; K (R.S.), Ramsay and Shields constant;* T_b, *b.p. °A;* t_c, *critical temperature °C.;* T_c, *critical temperature °A;* V_m, *molecular volume.*

The latent heats of evaporation are given in Table I. They were calculated from the vapor pressure curves by means of the Clapeyron equation. Boiling points are also given in Table I.

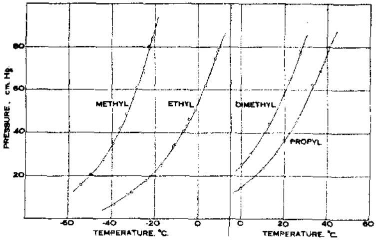

FIG. 3. *Vapor pressure of methyl, ethyl, propyl and dimethyl acetylenes.*

Discussion

The constants determined for all the hydrocarbons investigated in this laboratory are compiled in Table I. In the discussion of the acetylenes the properties will, in general, be taken up in the order in which they appear in the table.

It is seen that the melting points of the acetylenes are, for compounds containing the same number of carbon atoms, higher than those of either the paraffins or olefines. Unsaturation in the acetylenes apparently accounts for their higher melting points, and furthermore symmetry of the molecule seems to play an important role in governing this property, as may be seen in the case of the isomers ethyl acetylene and dimethyl acetylene.

It will be observed, when the more additive boiling points (Column 2), molecular latent heat of evaporation (Column 3) and critical temperature (Column 9), are compared, that for compounds containing the same number of carbon atoms there is a decrease in all these properties in the paraffin and olefine series as compared with the acetylene series. However, for the acetylenes that are still lower in molecular weight, these values are distinctly higher than those for their corresponding compounds in the other two series. This apparent anomaly makes it possible to classify the acetylenes in a group by themselves, separate from the paraffins and olefines. This is more evident as other properties are considered and is due to the fact that the acetylene linkage is of a more definitely pronounced polar character.

That all these substances are in the liquid state, in an unassociated normal condition, is shown by the values of the Ramsay and Shields constants (Column 7), although the values obtained for the acetylenes seem to be consistently low.

When considering the total surface energy (Column 6) in relation to the first few members of an homologous series, two types of compounds may be separately classified. There is first that in which no polar group is attached to the molecule, such as the paraffins and olefines listed above. Second, there is a large number of compounds such as the alcohols and fatty acids which possess a highly polar group attached to one end of the molecule.

As can be seen from the data assembled in Table I, the total surface energies of those compounds belonging to the first class increase slightly until a more or less limiting value is reached.

In compounds of the second type an altogether different result is observed. Several homologous series of this kind have been investigated and Hunten and Maass (5) found, when studying an homologous series of fatty acids, that the total surface energy falls off very rapidly as the series is ascended and reaches a normal value for all the higher members of the series. This same result has been found for other series whose members contain a polar group.

When the total surface energies of the acetylenes are compared it is seen at once that they follow this general tendency. This evidence points to the fact, not encountered in the paraffin and olefine series, that the acetylene linkage, a triple bond, exerts a decidedly polar influence on the molecule.

In general the more symmetrical the molecule the greater is its attraction for molecules of the same species. Thus it would be expected that dimethyl acetylene, being the symmetrical isomer of ethyl acetylene, would have a higher surface tension at the boiling point (Column 5). This theory is apparently not supported by the data obtained for the acetylenes. However, in this particular case the molecule is symmetrical around a group that is highly polar, compared to the polarity of the olefines, and since the property of surface tension is particularly sensitive to this factor it may well be due to this that the symmetrical dimethyl acetylene has a smaller surface tension at the boiling point.

Consequently all properties depending on surface tension, owing to the symmetry of the molecule, *i.e.*, the shielding effect of the hydrogen atoms surrounding the triple bond, are lower for dimethyl acetylene than for ethyl acetylene. As will be seen later this symmetry seems also to make the molecule appear less unsaturated. Thus it is not surprising to find that the parachor, which is directly related to surface tension, should have a value considerably lower for the symmetrical isomer and also lower than the theoretical value, which does not take into account certain "strain" factors apparently caused by symmetry of the molecule.

Confining the discussion to an empirical conception of these compounds, the acetylenes, and dimethly acetylene, in particular, may be considered from another point of view. If the molecular volume at the boiling point (Column 11) is considered to give a comparative value of the actual volume of the molecules, then this volume has been found to be an additive and constitutive property from the point of view of definite values assigned to the elements and their mode of linkage. Thus carbon and hydrogen in all organic compounds have fairly definite values where the carbon has four ordinary linkages. Unsaturation requires the addition of a constant value depending on whether the unsaturation is of an ethylene or acetylene type. The latter has the larger value, and the greater the extent of unsaturation the greater is the difference between the molecular volume of the compound and that given by the straight additive values of the atoms of the elements involved.

The molecular volumes of the acetylenes, using Kopp's constants where C = 2H = 11, are given in Table II and compared with their experimental values determined from the density at the boiling point.

It is seen that the difference in volume caused by the triple bond is greatest for acetylene and falls off rapidly as the series is ascended. That the value for dimethyl acetylene should be much less than that for the unsymmetrical isomer is indicative of the fact that the molecule of dimethyl acetylene is rendered less unsaturated by the symmetry of its structure.

TABLE II

MOLECULAR VOLUMES OF THE ACETYLENES

—	Kopp's	Experimental	Diff.
Acetylene	33.0	41.9	8.9
Methyl acetylene	55.0	59.7	4.7
Ethyl acetylene	77.0	80.8	3.8
Dimethyl acetylene	77.0	78.6	1.6
Propyl acetylene	99.0	101.3	2.3

This evidence, obtained from an entirely empirical point of view, agrees with, and moreover substantiates, the same conclusions drawn from a consideration of the calculated values obtained from surface tension measurements.

Finally, it may be said that all the properties of dimethyl acetylene, in relation to its own series and to members of the paraffin and olefine series, point to the fact that its behavior is greatly influenced by its polarity and molecular strain. All the properties that would be influenced by polarity of the molecule are seen to manifest this characteristic in the acetylene series by their divergence from the general tendencies observed in the paraffin and olefine series.

In conclusion, the acetylenes as a class of compounds stand out separately and distinctly from the paraffins and olefines, as far as the physical properties and their interpretation are concerned.

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β-[3-NITROPHENYL]-β-[ANTHRON-(9)-YL-(10)]-PROPIONIC ACID AND ITS DERIVATIVES[1]

BY ALEXANDRE VACHON[2], PAUL E. GAGNON[3] AND JOHN KANE[4]

Abstract

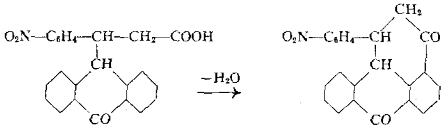
β-[3-Nitrophenyl]-β-[anthron-(9)-yl-(10)]-propionic acid, unlike β-phenyl-β-[anthron-(9)-yl-(10]-propionic acid (2, 3), was not found to undergo cyclisation to form a hydrindone derivative when treated with aluminium chloride. Treatment with concentrated sulphuric acid caused the formation of β-[3-nitrophenyl]-β-[(10)-oxy-anthron-(9)-yl-(10)]-propionic acid. The lactone of this acid was then formed together with Bz-1'-oxy-3'(3-nitrophenyl)-1-9-benzanthrone.

The following compounds have been prepared, as far as the authors are aware, for the first time: 3-nitrobenzylidene-dimethyl-malonate; the dimethyl and diethyl esters of β-[3-nitrophenyl]-β-[anthron-(9)-yl-(10)]-isosuccinic acid; β-[3-nitrophenyl]-β-[anthron-(9)-yl-(10)]-propionic acid; the chloride and ethyl ester of β-[3-nitrophenyl]-β-[anthron-(9)-yl-(10)]-propionic acid; β-[3-nitrophenyl]-β-[(10)-oxy-anthron-(9)-yl-(10)]-propionic acid and its lactone; and Bz-1'-oxy-Bz-3'(3-nitrophenyl)-1-9-benzanthrone.

Introduction

In previous papers (2, 3) it has been shown that by cyclisation of β-phenyl-β-[anthron-(9)-yl-(10)]-propionic acid or its chloride, hydrindone derivatives can be prepared.

Theoretically, it might be expected that β-[3-nitrophenyl]-β-[anthron-(9)-yl-(10)]-propionic acid (I), or its chloride, would not give rise to hydrindone derivatives, owing to the presence of the nitro group, since it is difficult to bring about cyclisation of nitropropionic acid (7). The authors were of the opinion that a benzanthrone derivative (II) would be formed instead (1).



(I) (II)

Accordingly, β-[3-nitrophenyl]-β-[anthron-(9)-yl-(10)]-propionic acid and its chloride, which have not hitherto been described, were prepared by a method similar to that used in the preparation of β-phenyl-β-[anthron-(9)-yl-(10)]-propionic acid (2, 3). The acid and its chloride were then treated with sulphuric acid and with aluminium chloride in attempts to bring about cyclisation.

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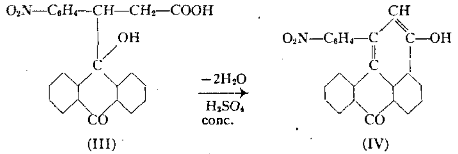
Contribution from the "Ecole Supérieure de Chimie", Laval University, Quebec, with assistance from the National Research Council of Canada. This paper is based on a thesis presented by John Kane in partial fulfilment of the requirements for the degree of D.Sc. at Laval University.
[2] *Director of the "Ecole Supérieure de Chimie", Laval University.*
[3] *Professor of Chemistry, Laval University.*
[4] *Graduate Student, Laval University.*

The acid was obtained by condensation of 3-nitrobenzaldehyde with dimethyl-malonate, and by further condensation of the product with anthrone. The dimethyl ester of β-[3-nitrophenyl]-β-[anthron-(9)-yl-(10)]-isosuccinic acid thus formed gave rise, on hydrolysis, to β-[3-nitrophenyl]-β-[anthron-(9)-yl-(10)]-propionic acid, which, on treatment with phosphorus pentachloride, yielded the chloride. The diethyl ester, which is cheaper, may also be used.

β-[3-Nitrophenyl]-β-[anthron-(9)-yl-(10)]-propionyl chloride is a white solid; m.p. 147° C. It is much more stable than the corresponding β-phenyl-β-[anthron-(9)-yl-(10)]-propionyl chloride. When dissolved in anhydrous carbon disulphide and treated with pure aluminium chloride, it does not give rise to hydrindone derivatives, as does the other; it remains unaltered. The nitro group in the para position renders the elimination of a molecule of hydrogen chloride more difficult.

Nitrobenzene was also used as solvent, but on heating the solution to temperatures greater than 100° C., hardly any hydrogen chloride was evolved, and a large quantity of resins was formed. Benzene was not used as solvent as it was assumed that this chloride, like β-phenyl-β-[anthron-(9)-yl-(10)]-propionyl chloride, would react with this solvent.

β-[3-Nitrophenyl]-β-[anthron-(9)-yl-(10)]-propionic acid is more stable than β-phenyl-β-[anthron-(9)-yl-(10)]-propionic acid. With sulphuric acid, it was impossible to obtain hydrindone derivatives or the expected benzanthrone derivative. Two other products were formed, however—a lactone and an oxy-benzanthrone (IV). In order to effect cyclisation, it was necessary to heat the sulphuric acid mixture at 150° C., and there was then a strong evolution of sulphur dioxide. β-[3-Nitrophenyl]-β-[anthron-(9)-yl-(10)]-propionic acid was oxidized to β-[3-nitrophenyl]-β-[(10)-oxy-anthron-(9)-yl-(10)]-propionic acid (III), as it contains an easily oxidizable tertiary carbon (6). The oxy-acid immediately lost one or two molecules of water to give rise to the lactone or to the oxy-benzanthrone.



(III) (IV)

The oxy-acid was easily prepared by oxidizing β-[3-nitrophenyl]-β-[anthron-(9)-yl-(10)]-propionic acid at room temperature with the calculated amount of potassium permanganate, and also by hydrolysis of the lactone. When treated with concentrated sulphuric acid the oxy-acid yielded the lactone and Bz-1′-oxy-Bz-3′(3-nitrophenyl)-1-9-benzanthrone.

The constitution of the lactone was established by hydrolyzing it, and also by oxidizing it with potassium permanganate in alkaline solution. The only products obtained were 3-nitrobenzoic acid and anthraquinone.

Bz-1′-oxy-Bz-3′(3-nitrophenyl)-1-9-benzanthrone, oxidized under the same conditions, did not yield anthraquinone, but gave rise to acidic products. The oxy-benzanthrone is a red compound which can exist in two tautomeric forms.

It is soluble in alcohol and is yellow in acid solution and violet in alkaline solution. It is a good indicator; the change in color from violet to yellow occurs when the pH value is 4.8 (determined with hydrogen electrode).

Experimental

3-Nitrobenzylidene-malonic and β-[3-Nitrophenyl]-β-[anthron-(9)-yl-(10)]-isosuccinic Esters

3-Nitrobenzylidene-dimethyl-malonate. The reaction was carried out at room temperature, as Knoevenagel's method (4) for the 4-nitro compound gave lower yields. Dimethyl-malonate (22 gm.), and 3-nitrobenzaldehyde (26.4 gm.), were placed in a flask, which was well stoppered, and left for nine days at room temperature. After three days the solution was clear. Later 3-nitrobenzylidene-dimethyl-malonate separated slowly, and after nine days the contents of the flask was solid. After crystallization from alcohol and washing with ether, 33 gm. of 3-nitrobenzylidene-dimethyl-malonate was obtained; m.p., 99–100° C.; (theory, 44.1 gm.). Yield, 75%. Calcd. for $C_{12}H_{11}O_6N$: C, 54.31; H, 4.18; N, 5.28%. Found: C, 53.79; H, 4.21; N, 5.15%.

3-Nitrobenzylidene-diethyl-malonate. This substance was readily obtained by Kötz's method (5), by condensing, in the presence of piperidine, equi-molecular quantities of 3-nitrobenzaldehyde and diethyl-malonate. From 125.8 gm. of diethyl-malonate, 186.9 gm. of 3-nitrobenzylidene-diethyl-malonate was obtained; m.p., 75–76° C. Yield, 81%.

Dimethyl ester of β-[3-nitrophenyl]-β-[anthron-(9)-yl-(10)]-isosuccinic acid, $O_2N \cdot C_6H_4 \cdot CH(C_{14}H_9O)CH(COOCH_3)_2$. Anthrone (8 gm.), crystallized from benzene and free from acetic acid, 12 gm. of 3-nitrobenzylidene-dimethyl-malonate, 80 cc. of anhydrous methyl alcohol and few drops of piperidine were heated in a flask, provided with a calcium chloride tube, on the water bath for five to six hours.

After cooling, the solid mass was filtered and washed with a little methyl alcohol. The ester was then almost pure. It crystallizes from acetic acid in colorless lens-shaped crystals; m.p., 188–189° C. Yield, 16 gm. (theory, 19 gm.), *i.e.*, 84%.

The ester is slightly soluble in methyl and ethyl alcohols and fairly soluble in acetic acid. Concentrated sulphuric acid dissolves it, forming a reddish solution which turns violet after a few days. Calcd. for $C_{26}H_{21}O_7N$: N, 3.05% Found: N, 3.08%.

Diethyl ester of β-[3-nitrophenyl]-β-[anthron-(9)-yl-(10)]-isosuccinic acid $O_2N \cdot C_6H_4 \cdot CH(C_{14}H_9O) \cdot CH(COOC_2H_5)_2$. Anthrone (31.5 gm.), free from

acetic acid, 50 gm. of 3-nitrobenzylidene-diethyl-malonate and 80 cc. of anhydrous methyl alcohol were heated in a flask (reflux condenser) on a water bath, and a few drops of piperidine were added. The anthrone dissolved rapidly; 10 min. later, a few additional drops of piperidine were added and the flask was left on the water bath for another half hour.

The hot solution was rapidly filtered with suction and cooled to 0° C. On stirring, the product crystallized. It was filtered off, and washed with ether. The product was colorless. From 189 gm. of anthrone (six preparations), 393.5 gm. of the diethyl ester of β-[3-nitrophenyl]-β-[anthron-(9)-yl-(10)]-isosuccinic acid was obtained. Yield, 83%. Recrystallized from methyl alcohol, it melted at 135–137° C. The ester is soluble in benzene, ether and acetic acid. Calcd. for $C_{28}H_{25}O_6N$: N, 2.87%. Found: N, 3.03%.

β-[3-Nitrophenyl]-β-[anthron-(9)-yl-(10)]-propionic Acid

Preparation from the dimethyl ester (10). The dimethyl ester of β-[3-nitrophenyl]-β-[anthron-(9)-yl-(10)]-isosuccinic acid (10 gm.), 30% sulphuric acid (58 cc.) and glacial acetic acid (23 cc.) were heated in a flask (reflux condenser) for eight days at 120–125° C. After cooling, the mixture was poured into 300 cc. of water. The insoluble acid was filtered off, pulverized and washed with water; 8 gm. of a yellowish product was obtained. It was dissolved in a hot solution of sodium bicarbonate and, after cooling, the solution was filtered and acidified with dilute sulphuric acid. The precipitated acid was filtered off, washed with water and recrystallized from acetic acid.

The acid is readily soluble in concentrated ammonia, fairly soluble in methyl alcohol and acetic acid and slightly soluble in ethyl alcohol. From ethyl alcohol the pure acid crystallized in colorless rectangular crystals; m.p., 206–207° C.

The acid can be readily oxidized with potassium permanganate in alkaline solution, as will be shown below. It is fairly stable towards alkalies. Treatment with a boiling solution of potassium hydroxide (30%) merely dissolves it. Calcd. for $C_{23}H_{17}O_5N$: C, 71.29; H, 4.42; N, 3.61%. Found: C, 71.16; H, 4.41; N, 3.60%.

Preparation from the diethyl ester. The diethyl ester of β-[3-nitrophenyl]-β-[anthron-(9)-yl-(10)]-isosuccinic acid (80 gm.), 30% sulphuric acid (430 cc.) and glacial acetic acid (172 cc.) were heated to boiling in a flask (reflux condenser). The ester melted and separated into small drops. After heating for three days the droplets solidified. The mixture was boiled for another day. (It was noted that if heating was prolonged (six or seven days) an impure acid, which could not be purified by many crystallizations from acetic acid, was obtained.) After cooling, the contents of the flask was poured into water and the insoluble acid was filtered off, pulverized, washed with water and dissolved in a hot saturated solution of sodium bicarbonate. After cooling, the solution was filtered and acidified with dilute sulphuric acid. The precipitated acid was filtered off, crystallized from acetic acid and washed with alcohol; m.p., 206–207° C. Yield, 45 gm. (74%).

This acid was proved identical with that obtained by hydrolysis of the *dimethyl ester of β-[3-nitrophenyl]-β-[anthron-(9)-yl-(10]-isosuccinic acid* by a mixed melting point determination.

β-[3-Nitrophenyl]-β-[anthron-(9)-yl-(10)]-propionyl chloride, $O_2N . C_6H_4 CH(C_{14}H_9O)CH_2 . COCl$. Pure β-[3-nitrophenyl]-β-[anthron-(9)-yl-(10)]- propionic acid (30 gm.), phosphorus pentachloride (17 gm.) and anhydrous carbon disulphide (200 cc.) were boiled in a flask, fitted with a reflux condenser and a calcium chloride tube, for two hours. The acid did not dissolve, but there was a strong evolution of hydrogen chloride, and crystals of the chloride were deposited; the unchanged acid remained uniformly dispersed in the solvent. After cooling, the chloride was filtered off and washed with anhydrous carbon disulphide. Yield, 29.3 gm. (93%). The chloride is slightly soluble in carbon disulphide, which is the best solvent for purifying it, the chloride being practically insoluble in most organic solvents. It forms colorless prisms; m.p., 147° C. Calcd. for $C_{23}H_{16}O_4NCl$: Cl, 8.74%. Found: Cl, 8.64%.

Ethyl ester, $O_2N . C_6H_4 . CH(C_{14}H_9O)CH_2 . COOC_2H_5$. β-[3-Nitrophenyl]-β-[anthron-(9)-yl-(10)]-propionyl chloride (5 gm.) and anhydrous ethyl alcohol (80 cc.) were boiled in a flask (reflux condenser) for 1½ hr. and left at room temperature overnight. The alcohol was distilled off and the residue dissolved in ether. On evaporation of the solvent, the ethyl ester crystallized out. It was recrystallized from alcohol and obtained in colorless prisms; m.p., 107–109° C. Yield, 4.5 gm. (88%). Calcd. for $C_{25}H_{21}O_5N$: N, 3.37%. Found: N, 3.28%.

β-[3-Nitrophenyl]-β-[(10)-oxy-anthron-(9)-yl-(10)]-propionic Acid Lactone

Preparation. Dried and well pulverized β-[3-nitrophenyl]-β-[anthron-(9)-yl-(10)]-propionic acid (25 gm.) and concentrated sulphuric acid (250 cc.) were heated carefully in a flask and well shaken. The acid dissolved, forming a yellowish solution which became reddish at 115° C. and violet at 140° C. The mixture was heated to 150° C. and kept at that temperature for five minutes. Sulphur dioxide was evolved.

The hot solution was poured on to cracked ice. A brownish product precipitated. The mixture was diluted to three litres with water, heated to about 80° C. to coagulate the solid, cooled to room temperature, and filtered. The acid filtrate gave a dark violet color with alkalies. This color was due, as will be shown later, to the presence of a trace of Bz-1′-oxy-Bz-3′(3-nitrophenyl)-1-9-benzanthrone. The strongly colored product which remained on the filter paper was dried. It weighed 23.8 gm. Many extractions were made with 800-cc. portions of hot chloroform. Some of the solid dissolved. The dried residue (about 7 gm.) was identified as β-[3-nitrophenyl]-β-[anthron-(9)-yl-(10)]-propionic acid. The chloroform solution was left to cool, and about 1 gm. of red crystals separated. They were filtered off and dried. They will be described later.

The filtrate was evaporated to dryness and 15 gm. of a product, still slightly colored by a trace of the red compound, was obtained (theory, 24.8 gm.).

Yield, 60%. If account is taken of the unaltered propionic acid (7 gm.), the yield was 88%. After crystallizing once from acetic acid and washing once with ether, the product was white and melted at 267–268° C.

The temperature and the time of heating employed seem to be the optimum for obtaining the best yield. In order that the lactone be formed, it is necessary to heat at a temperature of at least 140° C. and if the temperature is raised to 150° C., the time of heating must not be long. For instance, when the heating period was 12 min., the yield was only 46%, and when 30 min., carbonization took place. Many preparations were made under different conditions, not only to obtain a large quantity of the lactone, but also in attempts to increase the yield of the red compound, but only a very small quantity of the red substance was formed each time.

The lactone is slightly soluble in most of the organic solvents. It is insoluble in ether, carbon tetrachloride, carbon disulphide and methyl alcohol. It is very slightly soluble in ethyl alcohol, and somewhat more soluble in chloroform, toluene and acetic acid. The lactone is very readily decomposed by caustic alkalies, yielding anthraquinone. However, it was possible to hydrolyze it with a very dilute solution of potassium hydroxide, and so obtain the corresponding β-[3-nitrophenyl]-β-[(10)-oxy-anthron-(9)-yl-(10)]-propionic acid, as shown below. Calcd. for $C_{23}H_{16}O_6N$: C, 71.66; H, 3.92%. Found: C, 71.52; H, 3.90%.

Bz-1′-oxy-Bz-3′(3-nitrophenyl)-1-9-benzanthrone

In the preparation of the lactone, which was just described, traces of the red compound were separated by filtration. Analysis indicated the formula $C_{23}H_{13}O_4N$. From its properties, the oxidization products and its formation from the oxy-acid, it is believed to be a benzanthrone derivative which can exist in two tautomeric forms.

The red compound is fairly soluble in ether, acetic acid and alcohol. From the last two solvents it separates in crystals of the shape of a convex lens; m.p., about 305° C. With concentrated sulphuric acid, a violet solution (red by transmitted light) is obtained, which turns yellow when water is added. Its value as an indicator has been mentioned in the introduction. Calcd. for $C_{23}H_{13}O_4N$: C, 75.18; H, 3.57%. Found: C, 74.80; H, 3.51%.

Oxidation of the Lactone with Potassium Permanganate

The lactone (5 gm.), pulverized potassium hydroxide (3.4 gm.) and water (200 cc.) were heated in a flask (reflux condenser) on the water bath and 9.5 gm. of potassium permanganate was slowly added. At first the permanganate was reduced fairly rapidly, but after three hours the solution was only very slowly decolorized. The flask was left on the water bath for about 17 hr. The hot mixture was filtered with suction. The residue was washed with hot water, treated with dilute hydrochloric acid and extracted with chloroform. The solution was washed with water and dried with anhydrous calcium chloride. The solvent was then distilled off; 2 gm. of a yellowish product remained. The product separated from acetic acid in long needles; m.p.,

277–278° C. It was identified as anthraquinone by a mixed melting point determination. The filtrate was evaporated to small volume, acidified with dilute hydrochloric acid and extracted many times with ether. The ether was distilled off and about 2 gm. of a resinous product was obtained. It was very soluble in ether and alcohol and very slightly soluble in petroleum ether and benzene. It was difficult to purify it by crystallization. When heated carefully, some sublimed and formed long colorless needles. These were soluble in hot water and, after one crystallization from water, melted at 140–141° C. This substance was proved identical with 3-nitrobenzoic acid by a mixed melting point determination and by analysis. Calcd. for $C_7H_5O_4N$: C, 50.28; H, 3.01%. Found: C, 50.32; H, 3.12%.

Oxidation Products of Bz-1'-oxy-Bz-3'(3-nitrophenyl)-1-9-benzanthrone

Only a very small quantity of the oxy-benzanthrone was obtained in the preparation of the lactone. However, it was possible to prove that when the oxy-benzanthrone was oxidized under the same conditions as those used in the oxidation of the lactone, anthraquinone was not formed. The largest portion of the product obtained was acid in nature, indicating that it was a benzanthrone derivative. It was impossible to carry the study of the oxidation products of this red compound any further.

β-[3-Nitrophenyl]-β-[(10)-oxy-anthron-(9)-yl-(10)]-propionic Acid

(a) Preparation from the acid. β-[3-Nitrophenyl]-β-[anthron-(9)-yl-(10)]-propionic acid (25 gm.), potassium hydroxide (6 gm.), potassium permanganate (6.8 gm.) and about one litre of water were placed in a flask and left at room temperature for 24 hr. The permanganate was completely reduced. The manganese dioxide was filtered off and washed with water. When the filtrate was neutralized, a white product separated. It was filtered off and washed with water until free from potassium chloride. It was very soluble in methyl alcohol, acetic acid and most organic solvents. However, it was possible to recrystallize it from a mixture of one part of methyl alcohol and four parts of water. Dried over anhydrous calcium chloride, it melted at 120° C. The product obtained is a hydrate containing one molecule of water. When it melts, the water is set free and, at a higher temperature, solidification again takes place.

The product was characterized by analysis after it had been crystallized from dilute alcohol and dried over anhydrous calcium chloride. The silver salt was prepared by the usual method.

β-[3-Nitrophenyl]-β-[(10)-oxy-anthron-(9)-yl-(10)]-propionic acid is much less stable than the β-[3-nitrophenyl]-β-[anthron-(9)-yl-(10]-propionic acid. It is readily decomposed by a boiling solution of potassium hydroxide (10%), yielding anthraquinone.

Heated with concentrated sulphuric acid at 150° C., the oxy-acid gave rise to the lactone and oxy-benzanthrone. Five grams was treated with 50 cc. of concentrated sulphuric acid and heated at 150° C. for five minutes. The mixture was poured on ice. The precipitate formed was filtered off, dried

and a portion was dissolved in hot chloroform. On cooling the solution, a fairly large quantity of red crystals separated. They were filtered off and identified as Bz-1′-oxy-Bz-3′(3-nitrophenyl)-1-9-benzanthrone by a mixed melting point determination.

The solution in chloroform was evaporated to dryness and the residue, purified by crystallization from acetic acid, was identified as the lactone by a mixed melting point determination. Calcd. for $C_{23}H_{17}O_6N \cdot H_2O$: C, 65.53; H, 4.54%. Found: C, 64.21; H, 4.55%. Calcd. for $C_{23}H_{16}O_6NAg$: Ag, 21.11%. Found: Ag, 21.21%.

(b) Preparation by hydrolysis of the lactone. The lactone (1 gm.) was treated with 0.15 gm. of potassium hydroxide in 5 cc. of alcohol (95%). The mixture was boiled for 15 min., cooled and diluted to 100 cc. with water. It was then filtered with suction. The dried residue (0.3 gm.) was identified as the starting material. The filtrate was acidified with hydrochloric acid (10%), and the precipitated acid was filtered off, washed with water and dried over anhydrous calcium chloride (0.6 gm.). It was dissolved in a hot saturated solution of sodium bicarbonate. After cooling, the solution was filtered and acidified again. This procedure was repeated twice. The acid was well washed with water and dried over anhydrous calcium chloride. It had the same properties as the acid prepared by the other method—it was very soluble in most of the organic solvents; it could be crystallized from a mixture of one part of alcohol to four parts of water; it was readily decomposed by a solution of potassium hydroxide (10%), yielding anthraquinone. When it was treated with concentrated sulphuric acid, Bz-1′-oxy-Bz-3′(3-nitrophenyl)-1-9-benzanthrone was formed. Calcd. for $C_{23}H_{17}O_6N \cdot H_2O$: C, 65.53; H, 4.54%. Found: C, 65.12; H, 4.21%.

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A PORTABLE GEIGER-MÜLLER TUBE COUNTER AS A DETECTOR FOR RADIOACTIVE ORES[1]

By G. M. Shrum[2] and Ronald Smith[3]

Abstract

A portable Geiger-Müller tube counter constructed for use as a geophysical instrument for locating radium-bearing ore bodies is described. Its chief characteristics are stability and low operating voltage, combined with great sensitivity to gamma radiation. The weight, using standard batteries, is about 55 lb. It is self-contained and can be used in rough country and in wet weather. Other uses suggested are in connection with natural gas wells, radium springs, mining operations, and for the location of lost radium needles and preparations used in hospitals.

Introduction

As the rich radium-bearing ore deposits in the Great Bear Lake district were discovered as outcroppings without the aid of scientific instruments, it seems reasonable to assume that other deposits might be discovered if suitable prospecting devices for penetrating the glacial overburden were obtainable. The gold-leaf electroscope is about the only instrument which has hitherto been available for this purpose. Although it is simple and portable, it is a very unsatisfactory instrument for field work, because of its fragility, the necessity for keeping it free from moisture, and the time required for taking readings. Any instrument developed to replace the electroscope should meet the following requirements: (*a*) It should be rugged enough to withstand field conditions; (*b*) the sensitivity should not be less than that of a laboratory gamma ray electroscope; (*c*) the time required for readings should be a minimum; (*d*) the instrument should be so simple in operation that it could be used effectively by persons with no special training in physics. By utilizing the principle of the Geiger-Müller counting tube an instrument has been developed which under actual test has fulfilled these requirements.

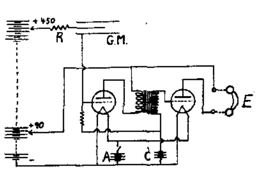

FIG. 1. *Diagram showing the simple amplifying circuit used with the portable Geiger-Müller counting tube.*

The Geiger-Müller Counting Tube

The electron counting tube developed by Geiger and Müller (5) and others (3, 6, 7, 11) for detecting gamma rays is well known. *Fig. 1 is a diagram of the arrangement* usually adopted for the operation of these counting tubes. The tube *G.M.* has as essential parts concentric electrodes placed so that the cylindrical brass cathode surrounds the fine tungsten wire anode.

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• *Contribution from the Physics Laboratory, University of British Columbia, Vancouver, Canada.*
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It is filled with a gas at 6 to 8 cm. pressure. A battery maintains the cylinder at a high negative potential with respect to the wire. R, a resistance of the order of 10^9 ohms, is placed in series with the tube. A two- or three-stage vacuum-tube amplifier with ear phones, E, or loud speaker is used to detect current surges in the circuit. When the potential across the tube is 1000-1500 volts, discharges occur. When amplified, these produce sharp clicks in the receiving set. The frequency of these discharges is greatly increased if a small quantity of radium is placed close to the tube. The residual or accidental discharges in the absence of radium are due to cosmic rays and stray gamma rays from the earth, the walls of the room or the parts of the apparatus. There is a definite minimum operating potential which varies with the dimensions of the wire and the surrounding metal cylinder, and on the nature and pressure of the gas in the tube. Above this minimum or threshold voltage there is a range, the breadth of which varies with the tube, in which the count per minute is fairly constant. This range (voltage plateau) is one in which the tube must be operated. At still higher voltages spurious discharges take place, the number increasing with the applied voltage.

The extreme sensitivity of these tubes to gamma radiation makes them very useful as detectors of radioactive substances. However, for field work a constant d.-c. potential of 1000-1500 volts offers many difficulties. Therefore it was necessary to develop a tube of great stability but with a lower operating potential.

A Low Operating Potential

In the development of a tube with a low operating voltage it was necessary to consider the three main factors which determine the threshold potential. They are: (1) diameter of the outside metal cylinder, (2) pressure of the gas in the tube, and (3) nature of the gas.

The variation of the threshold potential, V, with the diameter, b, of the metal cylinder is given in the formula (2)

$$X = \frac{2V}{a \log \frac{b}{a}},$$

where X, the electric field at the surface of the wire, remains constant for a constant wire diameter a; that is, the less b is, the lower will be V. However, the lowering of b is accompanied by a corresponding decrease of the effective area of the tube, thereby decreasing the sensitivity. In consideration of these factors a value for b of $\frac{1}{2}$ to $\frac{5}{8}$ in. was chosen. The voltage varies very little with the radius of the wire, fine tungsten or manganin wire being used throughout.

It has been shown (2) that the threshold potential of a Geiger-Müller tube changes with gas pressure in the same way as does the sparking potential of the gas used. Consequently it would be expected that a considerable decrease in potential should be obtained by lowering the pressure of the gas.

By this means the voltage may be reduced to 600 or 700 volts for air at 1 to 10 mm. pressure. However, it was found that at this low pressure spurious discharges were produced at voltages only a little above the threshold; thus it is evident that such tubes would be useless for work requiring accurate measurement. In addition the sensitivity (count per minute) was very appreciably decreased at these pressures, as Geiger and Müller have already pointed out (6).

The third variable, the nature of the gas, held the greatest promise of giving advantageous results. Gases other than air have been used successfully in these tubes, it being found that each gas requires in general a different operating voltage. The lowest potential reported by Geiger and Müller for any gas investigated by them was that for argon (6). Bosch and Klumb (1) and Schulze (10) have shown, however, that the inert gases are undesirable for use in these tubes. Using pure neon and helium they found that a continuous discharge took place at voltages slightly above the threshold. These results for helium and neon were confirmed by the writers and a similar effect with argon was observed. It was found, however, that when very small quantities of air were added to the argon, the continuous discharging ceased and the tube functioned normally. The threshold potential of a tube containing argon with traces of air was 360 volts at 1 cm. pressure. The reliable voltage range was about 50 volts—a value which compared favorably with that for the regular high voltage tubes.

When these argon tubes were sealed off, their characteristics sometimes changed over a period of time. When the brass was too well baked out before admitting the gas mixture the impurity seemed to be re-absorbed by the metal, and consequently the tube developed a tendency to discharge continuously. On the other hand, if no de-gassing was done before filling, the threshold voltage would gradually rise, apparently owing to a change in the composition of the gas mixture due to the emission of adsorbed gases. To obtain a condition under which the gas mixture would remain constant indefinitely, the tube was only partially de-gassed. It was then filled to the proper pressure with the gas mixture. It was not always possible to gauge accurately the amount of air admitted. When it was too small, sufficient additional gas could usually be obtained by heating the walls of the tube. From 7 to 10 mm. pressure of argon was found most satisfactory. This gave an operating potential of from 350 to 450 volts. Neon was found to behave in much the same way as argon, although under the same conditions the neon tubes showed a slightly lower threshold voltage.

The Complete Portable Outfit

A self-contained portable outfit comprising a tube and its auxiliary equipment was constructed.

The design of the tube adopted for field work is shown in Fig. 2. A tube sealed in glass was used to obviate the leakage and insulation difficulties encountered with metal–ebonite tubes. The outer electrode B, brass tubing

$\frac{5}{8}$ in. in diameter and from 1 to 6 in. long, is held in place by a copper wire *A* and the tungsten wire *T* sealed into one end of the Pyrex glass covering *D*. The central electrode, *M*, a fine tungsten or manganin wire, is suspended at one end from a glass hook, *G*, fused on to the glass wall, and at the other by a second tungsten wire sealed into the outer case. The final sealing, at *S*, is made at one end, eliminating all side projections and allowing the tube to be conveniently handled or packed. One end, T^1, is immersed in paraffin wax contained in another tube of slightly larger diameter; the series resistance, *R*, is also embedded in the paraffin. This outer covering acts not only as a protection against breakage but also prevents any electrical leakage at the terminal T^1, an important factor because fluctuations in the leakage current over the insulation are amplified and recorded as impulses. The whole is enclosed in a cylindrical wooden case, which serves as a protection against mechanical shock and also excludes the light, thereby preventing the photo-electric effects described by Rajewski (9) and Locher (8). The wooden case is mounted on the end of a hollow handle, through which the connection with the remainder of the apparatus is made by ordinary rubber-covered wire.

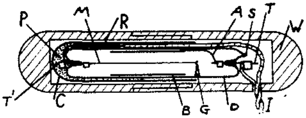

FIG. 2. *Diagram showing the mounting used for the portable Geiger-Müller tube. The paraffin wax,* P, *and glass tube,* C, *provide electrical insulation, while the wooden case,* W, *prevents breakage. The series resistance,* R, *is embedded in paraffin wax.*

The auxiliary equipment consists of a set of ten 45-volt batteries, a two-stage audio amplifier and a pair of ear-phones. The electrical arrangement is shown in Fig. 1, the plate voltage for the amplifier being tapped from the batteries used to supply the Geiger-Müller tube. The batteries and amplifier are placed in a waterproof case mounted on a standard pack-board, the whole weighing about 55 lb. This weight could be reduced by about 20 lb. by using specially designed batteries of light weight.

Sensitivity Tests

As with all small Geiger-Müller tubes, the number of residual or accidental discharges per minute due to cosmic rays and radioactive impurities undergoes a statistical fluctuation. One tube gave the following one-minute readings for 20 consecutive minutes: 10, 12, 11, 8, 10, 13, 9, 10, 9, 13, 6, 11, 14, 9, 9, 13, 7, 10, 10, 10. For longer periods, however, the fluctuations are much smaller, as the following five-minute readings obtained from the same tube show: 51, 54, 49, 50. Thus it may be seen that by taking five-minute readings an increase of five per minute in the count could be definitely detected. In case of doubt whether a high count is due to additional radioactive material in the neighborhood or to statistical fluctuation of the normal count, a longer reading period is required.

Laboratory Tests

Tests were made with radium and radium ore. A 53-gm. piece of pitchblende from Great Bear Lake containing 60% uranium oxide and about $\frac{8}{1000}$ mgm. of radium gave a count of 62 for five minutes when six feet from the detecting tube and 88 when three feet away. The zero count (no radium in the neighborhood) was 51 for a five-minute interval.

Eighty milligrams of radium in steel needles and enclosed in a small lead container was detected at a distance of 350 ft. taking only five-minute readings. Results of a test with this standard are shown in Table I. When these

TABLE I

RESULTS OF A TEST WITH 80 MGM. OF RADIUM IN STEEL NEEDLES IN A LEAD CONTAINER

Distance, ft.	100	150	200	250	300	350
Count, 5 min.*	265	139	99	82	64	55

* *Zero or normal count, 45.*

results are plotted with the count as ordinate and $\frac{1}{d^2}$ as abscissa, the inverse square law is obeyed within the limits of statistical error.

Field Tests

Field tests were carried out on Quadra Island in the Gulf of Georgia, from which samples of carnotite have been obtained. Fortunately a small section of this island had already been carefully surveyed by a party using a gold leaf electroscope, and the results were available for purposes of comparison. The electroscope gave some fairly high readings in certain areas and the survey indicated the possibility of commercial deposits of carnotite being found. Tests with the tube counter, however, showed that the only deposits were extremely small and localized. So far, subsequent exploration and development work on the property has confirmed the findings with this instrument.

Other Uses

It is well known that helium in varying proportions is a constituent of most natural gases. This helium is probably due to radioactive transformations in the neighboring rock structures. This suggests possibilities which could be investigated by lowering into the well the exploring tube of one of these instruments. It may be used also in the investigation of radium springs. And again in mining operations, one of these tubes could be lowered into a diamond drill hole, and thus be useful in locating any minerals which may be associated in some way with radioactive rocks.

Curtiss (4) has recently described a portable detector for locating small radium needles, etc. Judging from the various tests, it would appear that the instrument described here is probably 20 times as sensitive as that of Curtiss.

Acknowledgments

The authors wish to express their indebtedness to Dr. H. W. Riggs who kindly donated to the University a small quantity of radium, which has been most useful in these experiments; and to Dr. C. W. Prowd of St. Paul's Hospital, Vancouver, for the loan of radium needles.

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EXPERIMENTAL PROBABILITY[1]

By A. L. Clark[2]

Abstract

The work described in this paper is a continuation of that published previously (1).

The observations have now been carried to 250,000. The results are in substantial agreement with those already published. The theoretical probability is 0.3554 ±0.0005, and the new experimental value is 0.35553. The value of π is not changed by the later results. The numbers of sequences of different lengths have been recorded and compared with the calculated values. The agreement here is not very good.

In a previous paper an experimental investigation of probability was described and the very close agreement between calculated and experimental values was shown (1).

The work described in this paper is a continuation of that already discussed and the number of events has been increased from 100,000 to 250,000.

The method employed in all of the work is briefly as follows. Steel balls are dropped at random on to a steel plate pierced with holes in regular array. Since in these experiments, the array is triangular (hexagonal), the probability that a ball will pass through a hole without contact with the plate is

$$P = \frac{2\pi (R-r)^2}{d^2 \sqrt{3}} , \qquad (1)$$

where R and r are the radii of the holes and balls respectively and d is the distance between adjacent holes. There are certain corrections which must be applied to this value, and when these are applied the calculated value of the probability P is

$$P = 0.3554 \pm 0.0005. \qquad (2)$$

The experimental probability is the ratio of the number of passages of the balls without contact with the plate, divided by the total number of balls dropped. From the 100,000 events of the earlier paper the value of the experimental probability was 0.35558. By a process of averaging or interpolation carried out on the graph of the results, the value 0.35556 was taken as the best value obtainable from the data.

From the later experiments using 250,000 events, the final value of P is 0.35555. When P is plotted by 10,000's, the value obtained from the graph is 0.35550. If P is plotted by 25,000's on a larger scale, the value of P is slightly higher, 0.35553. There is therefore still a little uncertainty about the last figure.

It was pointed out in the previous paper that, while the agreement between the calculated and observed values of P is very good, it is really better than might be expected from the accuracy with which the various measurements

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of diameters and distances may be carried out. The newer work gives even closer agreement than was obtained before and it may be concluded, as was suggested, that the errors in the measurements of diameters and centre distances have neutralized each other to some extent.

Table I shows the values of the experimental probability and the fluctu-

TABLE I

Tabulated results

5,000's		10,000's		25,000's		50,000's		100,000's		To date	
P	δ	*P*	δ	*P*	δ	*P*	δ	*P*	δ	*P*	δ
.3516	−.0039									.3516	.0039
.3554	−.0001	.3535	−.0020							.3535	.0001
.3596	+.0041									.3555	.0000
.3516	−.0039	.3556	+.0001							.3546	−.0009
.3548	−.0007			.35460	−.00093					.3546	−.0009
.3614	+.0059	.3581	+.0026							.3557	+.0002
.3596	+.0041									.3563	+.0008
.3546	−.0009	.3571	+.0016							.3561	+.0006
.3456	+.0001									.3549	−.0006
.3654	+.0099	.3555	.0000	.35732	+.00179	.35596	+.00043*			.35596	+.00043
.3508	−.0047									.35555	+.00002
.3592	+.0037	.3550	−.0005							.35580	+.00027
.3512	−.0043									.35545	−.00008
.3538	−.0017	.3525	−.0030							.35533	−.00020
.3548	−.0007			.35396	−.00157					.35529	−.00024
.3574	+.0019	.3561	−.0006							.35543	−.00010
.3580	+.0025									.35558	+.00005
.3578	+.0023	.3579	+.0024							.35570	+.00017
.3548	−.0007									.35565	+.00012
.3542	−.0013	.3545	−.0010	.35644	+.00091	.35520	−.00033	.35558	+.00005	.35558	+.00005
.3622	+.0067									.35590	+.00037
.3598	+.0043	.3610	+.0055*							.35608	+.00105
.3440	−.0115									.35555	+.00002
.3564	+.0009	.3502	−.0053							.35558	+.00005
.3574	+.0019			.35596	+.00043					.35566	+.00013
.3460	−.0095	.3517	−.0038							.35528	−.00025
.3632	+.0077									.35558	+.00005
.3532	−.0023	.3582	+.0027							.35550	−.00003
.3632	+.0077									.35576	+.00023
.3458	−.0097	.3545	−.0010	.35428	−.00125	.35512	−.00041			.35543	−.00010
.3636	+.0081									.35569	+.00016
.3532	−.0023	.3584	+.0029							.35561	+.00008
.3580	+.0025									.35568	+.00015
.3472	−.0083	.3526	−.0029							.35544	−.00009
.3702	+.0147*			.35844	+.00291*					.35586	+.00033
.3506	−.0049	.3604	+.0049							.35571	+.00018
.3468	−.0087									.35547	−.00006
.3580	+.0025	.3524	−.0031							.35554	+.00001
.3574	+.0019									.35558	+.00005
.3518	−.0037	.3546	−.0009	.35292	−.00261	.35568	+.00015	.35540	−.00013*	.35550	−.00003
.3560	+.0005									.35550	−.00003
.3632	+.0077	.3596	+.0041							.35569	+.00016
.3488	−.0067									.35553	.00000
.3540	−.0015	.3514	−.0041							.35549	−.00004
.3568	+.0013			.35576	+.00023					.35552	−.00001
.3582	+.0027	.3575	+.0020							.35558	+.00005
.3530	−.0025									.35552	−.00001
.3498	−.0057	.3514	−.0041							.35540	−.00013
.3634	+.0079									.35557	+.00004
.3548	−.0007	.3591	+.0036	.35584	+.00031	.35580	+.00027			.35555	+.00002

Note:—*P is the experimental probability; δ is the fluctuation or the departure from the mean.*

ations about the mean, for series of different lengths. Since each page of the record book contains 1000 observations, it has been convenient to deal with the results by these 1000's. It might be, of course, that by selecting a different place, such as the middle of a page for the starting point of a series, slightly different and perhaps more interesting results might appear, but it is deemed better to deal with the observations in the more natural groupings which in a very real sense are random groupings.

These fluctuations are interesting and show clearly that, as the series length increases, the relative fluctuations decrease. There are exceptions, as it might be supposed would be the case. The word "expect" is hardly proper when discussing probability. Strictly speaking, one has no right to expect anything in probability experiments. One may predict (successfully or otherwise) but since anything may happen, expectation is barred.

The maximum values of the fluctuations are asterisked. These bring out very clearly what was pointed out in the earlier paper, *viz.*, the maximum relative fluctuations decrease very rapidly as the series length increases. One may not say that this will be the case always, since the improbable may happen at any time and upset all predictions, a point that can hardly be overemphasized.

As was done with the earlier results, the values of P by 100's for the entire series were noted and the number of times that each value appeared was plotted as a function of the value. Fig. 1 shows the result, and the resemblance to the ordinary probability curve is clearly seen. The fluctuations for this series length are seen as the departures of the values of P from the mean value which is indicated by the dotted line.

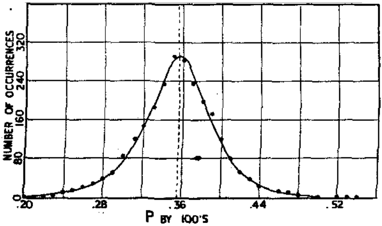

FIG. 1. *Distribution of values of P and fluctuations.*

The Value of π

As shown in the first paper, if P is known by experiment, π may be calculated from Equation (1). The value of π calculated in this manner for the first 100,000 events was 3.143 ± 0.005. The value of P for the second 100,000 is 0.35540, which is the *calculated value.* Hence if this value of P is used, the value of π should be 3.1416, the number used in calculating P. If Equation (1) may be regarded as a functional relation between P and π, π is proportional to P, and questions as to significant figures in the reverse calculation do not enter. The terminal value of P, 0.35555, and the averaged value 0.35553, differ so little from the value 0.35556 used in the earlier calculations, that the value of π is not affected sufficiently to show in the number of decimal places allowable.

Sequences

The numbers of sequences, or runs of 2, 3, 4, 5, etc., are calculated from

$$N = P^k n \ ,$$

where N is the number of sequences of length k, in a series of length n. Table II shows the calculated and observed values of N for values of k obtained from the experiment. The value of P is taken as 0.3555 which is near enough for this calculation. The agreement between the calculated and the observed values is on the whole not as good as shown in the previous paper for a series of 100,000. It is not surprising however that, even for long sequences where the number of occurrences is small, the agreement is not very good. That the earlier work showed better agreement must be regarded as fortuitous.

TABLE II

SEQUENCES

	By 50,000's						By 100,000's			For 250,000	
	Calc.	Observed					Calcd.	Observed		Calcd.	Obs.
		1st	2nd	3rd	4th	5th					
1's	17775	17798	17760	17756	17784	17790	35550	35558	35540	88875	88888
2's	6319	6501	5864	6236	3246	6022	12638	12259	9482	31595	29883
3's	2246	2483	2178	2336	1859	2226	4693	4655	4195	11232	11076
4's	799	834	655	720	503	675	1597	1489	1223	3993	3387
5's	284	346	220	253	157	230	568	572	410	1420	1207
6's	101	140	51	62	20	42	202	191	82	505	315
7's	36	66	8	15	3	10	72	74	18	179	102
8's	13	31	2	5	0	1	26	33	5	64	39
9's	4	17	0	0	0	0	9	17	0	22	17
10's	1	9	0	0	0	0	3	9	0	8	9
11's	.6	5	0	0	0	0	1	5	0	3	5
12's	.2	3	0	0	0	0	.4	3	0	1	3
13's	.07	1	0	0	0	0	.1	1	0	.3	1

The sequences are shown for series lengths of 50,000, 100,000 and for the entire series of 250,000.

Discussion

It was stated rather positively in the earlier paper that 0.35556 might be taken as a very close approximation to the true value of P. In the light of the subsequent work this statement must be modified a little. The value of P rose to 0.35608 at 110,000 and fell to 0.35498 at 128,000. Thus even after a fairly long series has been completed and the conclusion has been reached that there will be no more fluctuation of a given amount, a few very low or very high values for a few thousands throw the value of P outside the limits deemed assignable. The third figure in the value of P has remained unchanged

however since the end of the 128th thousand and it is improbable that it would ever change again. The fourth figure, however, cannot safely be said to have reached a fixed value even for 250,000, although it is extremely probable that its value too will soon become constant. This fourth figure fluctuated up and down even in the fifth 50,000. Throughout the entire experiment, the observers were worried, first, lest the results were getting too large, and a few days later, lest they were getting too small. Several 1000's might be large, then several 1000's small, or again they might oscillate up and down. This is, of course, precisely what should happen, but during the course of the observations the feeling grows, one way and then the other, that something is wrong with the apparatus. Eventually, and no doubt not much further on, the fourth figure would become fixed, and then the fifth, and perhaps the sixth, if we had the courage and time to carry the series far enough. Of course one must be prepared for the improbable and the third figure or indeed the second might change.

There are slight grounds for suspicion that the areas of the holes in the portion of the plate more remote from the edges have been decreased slightly by the continued impact of the balls on to the plate. This reduction in no case is over 0.0001 in., and in most cases is much less. The effect on P is difficult to calculate without such a detailed study of the shapes and sizes of all of the holes as would involve an unjustifiable expenditure of time. It is safe to say, however, that such a distortion could not affect the value of P by more than 0.0002, which is well within the limits of error set. It may be, however, that the slight decrease in the experimental probability shown in the results is due to a slight decrease in available area of the holes.

The correction factor, 0.9983, applied in the earlier work, should be increased a little (but only a little) by improvement in the verticality of fall. It was noticed that whenever a fresh lot of balls was used, the departure from the vertical was always less. After careful investigation, it was found that if the balls were carefully cleaned and then given a very slight coating of grease, the paths were more nearly vertical and fewer balls departed from the vertical by more than a millimetre. The departure from the vertical is evidently caused by a slight rotary motion as the ball falls through the tube. Neither the actual direction nor the amount is predictable since both depend on the last contact of the ball with the dropping tube. The grease seems to cause a slight adhesion to the wall of the tube and much of the rotary motion is stopped. The balls always have a coating of grease when received from the makers.

The two effects mentioned in the last two paragraphs compensate each other to a considerable extent and the calculated value, 0.3554 ± 0.0005, need not be changed.

A point which came up through an unpublished criticism of the former paper is the appearance of the sequences of 12 and 13 in the first 50,000 trials. It was maintained that a 13 would appear once in 1,000,000 events, and therefore its appearance in the 49th thousand is an indication that

randomness had not been attained. In reply it is only necessary to ask in what 1000 could the appearance of a sequence of 13 be admitted. The criticism seems to imply that all 1000's are not alike and that perhaps after 1000 thousands have been run off, a peculiar one will appear in which a 13 might enter. That far-distant thousand seems to have magical properties. The current 1000 however is the only one in which an extraordinary or improbable phenomenon may appear and the far-distant one never arrives. We always have to do with the present.

Again, one has only to point to the remarkable agreement between experimental and calculated values of P to remove any doubt as to randomness. We have to conclude that the early appearance of long sequences was exactly what might occur. Finally, the failure of some of the experimental sequence values to agree with those calculated (Table II) must be explained in precisely the same way. We must be prepared to accept the appearance of the improbable or the non-appearance of the probable in limited probability experiments.

It may be argued that the early appearance of a long sequence might be due to a momentary relaxation of attention during the supposed sequence, with the result that a contact was not heard. Such a thing is possible but highly improbable. Neither will lack of experience do as an explanation, since 20,000 events were run off before the record was begun. Finally there is the possibility of a faint sound due to very light contact of the ball with the plate, so light as to escape notice*. This is possible, but the difference in the paths of the ball between hit and miss is so small as to make such an explanation of little value. In order to test this point the following was carried out. One of the balls used in the experiment was soldered to the top of the plate and a micrometer screw was arranged vertically on a swivel so that it might be swung on a vertical axis. The end of the micrometer screw therefore passed over the ball. Then by turning the micrometer screw, it could be made to approach the ball more and more closely, while the screw was constantly rotated on the swivel. Eventually the end of the screw touched the ball. The microphone gave substantially the same sound as when in the experiments a ball made glancing contact with the surface of one of the holes. By adjustment of the micrometer, the sound could be made heavy or light. It was found that the amount of motion of the screw to change from free passage to contact was very small indeed—*i.e.*, is of the order of 1/10,000 the radius of the hole—perhaps less. For a ball to make a contact which might escape detection its centre must lie in an annular ring whose area is so small that the probability of such an occurrence is approximately the same as the probability that a run of 13 might occur.

The experiment as a whole clearly indicates several things. (i) If randomness obtains, the values of the experimental and calculated probability agree

**A statement in the earlier paper may be a bit misleading. It was stated that there is no gradation of sound for grazing contacts. The character of the sound does not change but it is light or heavy according to the violence of the shock. There seem to be no "threshold" cases where one is undecided about the contact.*

very closely if the series is sufficiently extended. In an experiment where measurements of distance or length are involved, the experimental value gives a more accurate determination of the true probability than may be calculated from measurement, provided the series is long enough. (ii) Anything possible may happen at any time and extremely improbable phenomena may occur even in a moderately short series. (iii) The highly probable may fail to occur in a finite series. (iv) It is not permissible to draw conclusions from a few events and the practice of assigning probable values from a few observations may not be justifiable in some cases.

Acknowledgments

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REVIEWS AND NOTES

The Specific Heats and Densities of Some Hydrophobic Sols

Zwicky (8) has explained the anomalous specific heats of some electrolytes as a result of the strong electric field which exists in the neighborhood of the ions. The field polarizes the water around the ions and produces a hydrostatic pressure of many thousand atmospheres. Bridgman (1) has shown that the specific heat of water decreases with increase of pressure, and using his values Zwicky has calculated that this effect is large enough to account for the observed decrease in the specific heats of the solutions.

Müller (3) has suggested that this effect must also be present in colloidal solutions, and that it is large enough to explain the stability of sols which, on the basis of other theoretical considerations, contain particles too large to remain in suspension. Ostwald (4) and others have pointed out that this compression of the liquid surrounding the colloid particles should lead to an increase in the density of the solution.

Careful measurements were made of the specific heats of gold and copper hydrosols using the method of cooling. The specific heats of these sols (concentration about 40 mgm. in 100 cc. of solution) were found not to differ from that of water by more than one part in 400 over a temperature range from 25 to 30° C.

The densities of gold and copper hydrosols were measured by the method outlined by Washburn (6). In each of the samples tested the density was slightly less (not more than 4 parts in 100,000) than that calculated from the weight of the residue when the sol was evaporated. Paine's (5) experimental values for the densities of copper hydrosols (obtained in a study of their chemical constitution) are in agreement with calculated values to within 1 part in 100,000.

Free energy changes accompanying the formation of a colloidal solution were calculated by both Zwicky's and Webb's (7) methods. Even assuming particle charge of 1000 electrons and a concentration of 10^{13} particles per cc. (10^{-6} cm. radius), the compression effect is only about 10^{-8} calories per cc., and the polarization about 10^{-7} calories per cc. In accordance with these results measurable changes in specific heat and density are not to be expected.

The writers wish to express their sincere appreciation to Prof. E. F. Burton, of the Department of Physics, University of Toronto, for his helpful suggestions and advice throughout the investigation.

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STUDIES ON THE NATURE OF RUST RESISTANCE IN WHEAT

VI. EFFECT OF HYDROGEN ION CONCENTRATION, PHENOLIC COMPOUNDS, AND HOST EXTRACTS ON THE GERMINATION OF UREDINIOSPORES OF *PUCCINIA GRAMINIS TRITICI*, FORM 21[1]

BY J. ANSEL ANDERSON[2]

Abstract

Urediniospores of *Puccinia graminis tritici*, form 21, were sown on buffer solutions covering the range from pH 3 to pH 8. Maximum germination was obtained between pH 5.8 and 6.5, with indications that if the point of maximum germination could be determined with precision it would be found to lie in the neighborhood of pH 6.2.

The effect of buffered and unbuffered solutions of pure phenolic compounds on the germination of urediniospores was studied. Greater inhibition was obtained with unbuffered solutions owing to the additional effect of hydrogen ion concentration. In buffered solutions, at pH 6.0, germination was prevented by 45 p.p.m. of hydroquinone, 125 of *o*-cresol, 180 of anisic acid, 250 of benzoic acid, 340 of catechol, 400 of guaiacol and 600 of phenol. A number of other compounds were also studied. The inhibitory effects of compounds, of which the bactericidal effects are known, are fairly closely related to their bactericidal efficiency.

Investigations were made of the inhibitory effects on spore germination and the growth of germ tubes, of aqueous dilutions of 15, 10, 5 and 2.5% of the press-juice of Vernal, Khapli, Marquis and Little Club wheats. Statistical treatment of the data accumulated in ten series of determinations, in which a total of 160,000 spores was counted, showed that significant differences occurred between varieties and that variations in the total solid content and hydrogen ion concentration of the press-juice had no consistent effect upon the results. Although the results were not entirely clear-cut, it is concluded that the varieties fall in the following order with respect to increasing inhibitory effect of their extracts: Vernal, Marquis, Khapli and Little Club. Since Vernal and Khapli are resistant to form 21 whereas Marquis and Little Club are susceptible, the results fail to show any relationship between the effects of host extracts on the germination of urediniospores, or on the growth of germ tubes, and the rust resistance of the variety.

Introduction

It is generally believed that, with certain exceptions, seedling resistance to *Puccinia graminis tritici* is physiological rather than morphological in nature. Susceptibility and resistance appear to depend upon a balance or lack of

[1] *Manuscript received August 9, 1934.*
Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa, Canada, in co-operation with the Department of Field Crops, University of Alberta, Edmonton. Presented by R. Newton, F.R.S.C., at the meeting of the Royal Society of Canada, May, 1934.

[2] *Biologist, National Research Laboratories, Ottawa.*

balance between the physiological processes of the host and of the parasite. The complicated relations which exist between the various wheat varieties and biologic forms of the fungus preclude the possibility of any simple explanation of the differences between resistant and susceptible hosts. It seems probable that differences exist in the physiology of both the wheat varieties and the rust forms, and that the combinations and permutations of these give rise to the complicated relations which exist between host and parasite. If this be true, no explanation of the basis of rust resistance can be expected until adequate studies have been made of the physiological processes of a wide range of susceptible and resistant wheat varieties, and also of a large number of biologic forms of the parasite.

The difficulties of the investigation are increased enormously by the fact that the fungus cannot be cultured on artificial media. The spores will germinate and produce germ tubes, but growth is sustained for a day or so at most. In these circumstances it has not yet been possible to devise any adequate means whereby the effect on the parasite of any nutrient or growth inhibiting substances, which might be isolated from the host, can be studied.

Investigations which might have led to the development of such a test have been reported by Newton, Lehman and Clarke (7). They attempted to induce resistance in susceptible wheats by administering preparations of resistant varieties in three ways: (*a*) by adding them in various concentrations to water cultures of wheat seedlings; (*b*) by adding them to petri-dish cultures of wheat leaves; (*c*) by direct injection into leaves of plants growing in ordinary soil. Though the last two methods yielded indications of induced resistance, no clear-cut results were obtained.

Since that time Ezekiel (2) has published the results of an investigation designed, in part, to determine the relation between the ability of very dilute host extracts to support the growth of the germ tubes of urediniospores and the resistance of the host. The experiments, bearing directly on this discussion, were carried out mainly with Kanred and Mindum wheats and rust forms 18 and 19, to which the two wheats react reciprocally. In one experiment Little Club and form 1 were used in addition. In a further experiment 10 rust forms were tested, using extracts from Mindum wheat only. The urediniospores were cultured in hanging drops of 1% agar and a concentration of 0.25, 0.1, 0.01, 0.001 or 0.0001% of the host extract. Comparisons were made by measuring the length of the germ tubes after full growth had been attained. Interpretation of the results was complicated by the fact that the length of germ tubes depended upon the physiologic form used, the number of spores per unit volume of culture and the sample of spores used. Corrections were made for these three factors. The author concludes that: "Extracts prepared from wheat varieties differed in their ability to support the growth of physiological forms of *P. graminis tritici* in exact agreement with the respective resistance or susceptibility of the varieties to the various forms".

A careful study of the paper shows that the original data are extremely variable and that the methods used for correcting these for purposes of comparison are subject to serious objections. Nevertheless, the results appear to lend some support to the author's hypothesis.

Ezekiel's work appeared to open up possibilities for development of a test for the toxicity of host extracts which might be of considerable value as an adjunct to investigations of chemical differences between susceptible and resistant wheat varieties and their possible relation to rust resistance. In view of the variability of the results obtained by Ezekiel, and of the comparatively small differences between varieties, it was apparent that if conclusive results were to be obtained, further investigations would have to be made on such a scale that a body of data suitable for statistical analysis would be obtained. In these circumstances the highly developed and time-consuming technique of Ezekiel's germ-tube-measuring method precluded its use.

The alternative method of using more concentrated host extracts and measuring their inhibitive effect on spore germination rather than on the growth of germ tubes suggests itself at once. The method has certain objections. In the first place, it must be borne in mind that results obtained by germination counts cannot be directly compared with those obtained by measurements of germ tube length until it is shown that the inhibition of spore germination by higher concentrations of a compound or extract is directly related to the effect of lower concentrations on the growth of germ tubes. Thus similar results obtained by germination counts might serve to confirm Ezekiel's hypothesis, but contradictory results would not disprove it. However, since spores germinate on, and enter, the leaves of all wheat varieties (with certain possible exceptions due to the size and mechanism of stomatal openings), irrespective of whether the variety is resistant or susceptible, neither germination counts nor measurements of the growth of germ tubes can be said to simulate the conditions under which the fungus develops or fails to develop in the growing wheat plant. Thus there appears to be no *a priori* reason why one method should show a relation between the effect of extracts and varietal resistance and the other method should not.

In the second place, as early as 1902, Ward (10) reported that spores germinated equally well in extracts of the leaves of their own host species and of their antagonistic host species. This did not augur well for further investigations of spore germination. However the literature does not appear to contain any report of an investigation of spore germination on host extracts carried out on a scale capable of demonstrating definitely that varieties could be differentiated by this method. Such an investigation appeared to be worth undertaking.

The investigation was confined to studies of the effect of extracts of Vernal, Khapli, Marquis and Little Club wheats on the germination of urediniospores of a single rust form, No. 21. Preliminary experiments were made in 1932

on wheats grown in the field. Owing to the variability of the plants from day to day and to the fact that an insufficient number of determinations were made, the results obtained failed to demonstrate significant differences between varieties. However, the experience gained made it possible to plan the investigations carried out in 1933 which are reported in this paper.

Since Hursh (4) reported that the range of hydrogen ion concentrations over which maximum germination is obtained, differs for different rust forms, it was necessary to study the effect of hydrogen ion concentration on the germination of form 21 in order to determine what part this factor might play in the effect of host extracts.

The effect of dilute solutions of certain pure phenolic compounds upon the germination of urediniospores of form 21 was also investigated. It was hoped that time would be available for the study of the effect of a number of different compounds upon several rust forms. Since it has been shown that different bacteria differ in their resistance to different phenolic compounds (5) and that quite remarkable differences may exist in the resistance of different strains of the same bacterium (8), it seemed quite possible that various rust forms might show similar differences. If this proved to be true, the evidence accumulated might shed some light upon the characteristic ability of rust forms to attack certain wheat varieties and not others. Unfortunately it was not possible to complete this program and only a few preliminary studies of the reaction of spores of form 21 to phenolic compounds are reported.

Effect of Hydrogen Ion Concentration on the Germination of Urediniospores of Form 21

Materials and Methods

A pure strain of *Puccinia graminis tritici*, form 21, was obtained from the Dominion Rust Research Laboratory, Winnipeg. Stock cultures were maintained on Marquis seedlings grown in the greenhouse. No other rust forms were being cultured in this greenhouse at that time.

Clark and Lubs' (1) buffer solutions were used as a series of substrata covering the desired range, *viz.*: pH 3.0, phthalate and hydrochloric acid mixture; pH 4.0 to 6.2, phthalate and sodium hydroxide mixtures; pH 5.8 to 8.0, potassium acid phosphate and sodium hydroxide mixtures.

Spores from pustules four days old were used throughout the study, since preparatory investigations showed that spores from pustules of this age germinated most readily. Leaves bearing pustules were held over a dry petri dish and gently tapped. The spores thus collected were thoroughly mixed and a sample transferred to 5 ml. of the substrate, held in a syracuse dish, by dipping a camel-hair brush into the spores and tapping it above the dish. With practice a fairly even distribution of spores was obtained by this method. The dishes were stacked to prevent evaporation and incu-

bated in the dark for 15 hr. at 20° C. Germination counts were made under the microscope. In 1932 the determinations were carried out in duplicate, 500 spores being counted in each dish. In 1933 the determinations were carried out in quadruplicate and 250 spores were counted in each dish.

Experimental Results

The data obtained in 10 experiments carried out during 1932 and 1933 are reported in Table I, and a graph in which the mean percentage germination is plotted against pH is shown in Fig. 1. It was observed during the course of these experiments that the length of the germ tubes was roughly proportional to the number of spores germinating.

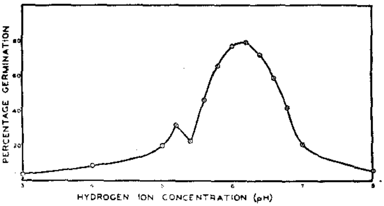

Fig. 1. *Effect of hydrogen ion concentration on the germination of urediniospores of form 21.*

Since Clark and Lubs' buffer solutions contain equal quantities of phthalate or phosphate, as the case may be, differences in pH being obtained by varying the concentration of acid and alkali, it seems reasonable to suppose that results obtained with these solutions will reflect only the effect of hydrogen ion concentration.

TABLE I

Effect of hydrogen ion concentration on germination

Substrate	pH	May 29, 1932, %	1932, %	1932, %	1932, %	May 29, 1933, %	June 22, 1933, %	May 31, 1933, %	June 1, 1933, %	June 28, 1933, %	July 19, 1933, %	Mean %
Phthalate + HCL	3.0	9	5	—	—	0	1	—	—	—	—	4
Phthalate + NaOH	4.0	18	15	—	—	1	3	—	—	—	—	9
Phthalate + NaOH	5.0	31	37	36	29	6	13	5	8	8	29	20
Phthalate + NaOH	5.2	—	—	49	26	—	—	8	15	41	50	32
Phthalate + NaOH	5.4	—	—	64	50	—	—	4	3	0	18	23
Phthalate + NaOH	5.6	—	—	66	37	—	—	40	67	29	34	46
Phthalate + NaOH	5.8	—	—	92	69	—	—	61	69	44	62	66
Phthalate + NaOH	6.0	71	85	91	82	65	79	67	82	77	70	77
Acid phosphate + NaOH	6.2	—	—	87	84	—	—	83	72	77	70	79
Acid phosphate + NaOH	6.4	—	—	84	68	—	—	67	79	68	68	72
Acid phosphate + NaOH	6.6	—	—	72	71	—	—	48	60	58	46	59
Acid phosphate + NaOH	6.8	—	—	63	47	—	—	34	40	40	27	42
Acid phosphate + NaOH	7.0	33	37	40	27	8	10	25	16	7	11	21
Acid phosphate + NaOH	8.0	9	8	—	—	4	1	—	—	—	—	6

Each percentage represents the count of 1000 spores.

Statistical Analyses

The data obtained in the six series of determinations covering the range from pH 5.0 to pH 7.0 were examined statistically by the method of analysis of variance. The total variance of the observed results was analyzed into portions due to differences in the general level of the results obtained in different series, to the average difference over all series between hydrogen ion concentrations, and to the remainder, representing differences in the relative percentage germination at various hydrogen ion concentrations in different series. The results of the analysis of variance are given in Table II.

TABLE II

Analysis of variance of data on the effect of hydrogen ion concentration on germination

Variance due to	Degrees of freedom	Sum of squares	Mean square
Series	5	6225	1245††
H ion concentrations	10	32324	3232††
Remainder	50	5455	109

†† *Significantly greater than the mean square due to remainder.*
In tables of analysis of variance throughout this paper
* *or* † *denotes that the mean square attains a 5% level of significance,*
** *or* †† *denotes that the mean square attains a 1% level of significance.*

The results show that there were significant differences in the general level of the results obtained in different series. This is attributed to differences in the spores used in different series. This portion of the variance, since its effect is equal at all hydrogen ion concentrations, may now be eliminated in further considerations of the data.

The average difference between hydrogen ion concentrations is shown to be highly significant. In order to evaluate the individual differences between the mean germinations for any two hydrogen ion concentrations, a criterion was set up as follows. The standard deviation of the mean of six determinations was calculated from the mean square due to the remainder and this multiplied by $\sqrt{2}$ and a factor gives the smallest difference between two means which can be judged significant for a 5% level of significance. The factor was obtained from published tables of "*t*" (3), the ratio of the observed difference between two means to the estimated standard error of the difference. The necessary difference for a 5% level of significance was found to be 12%. With this criterion in mind it is possible to evaluate the results of the investigation. It seems safe to assume that all hydrogen ion concentrations which give a percentage germination less than 67% (79%, the maximum germination obtained, minus 12%) fall outside the range of maximum germination. The range of maximum germination is therefore pH 5.8 to 6.5. The results also indicate that if the point of maximum germination could be determined with precision, it would be found to lie in the neighborhood of pH 6.2.

From the point of view of these studies the dip in the curve at pH 5.4 is of academic interest only. According to the criterion of the necessary differ-

than that for pH 5.2. However, inspection of the data will show that the low point occurs as a result of the four series of determinations made in 1933. It therefore seems just to examine the significance of this low point by analyzing only these four series. Application of the "*t* test" to the differences between the means of the percentage germination at pH 5.2 and 5.6 and the percentage germination at pH 5.4, in these four series, gave a value of *t* equal to 6.47. Since the required value for a 1% level of significance is only 5.84 it appears that the drop in the percentage germination at pH 5.4, found in the 1933 series, is definitely significant. No explanation for this apparent abnormality has yet been found.

The results obtained with form 21 do not support Hursh's hypothesis (4) that forms more limited in their host range are also more limited in their tolerance to extremes of hydrogen ion concentration. Hursh studied forms 11 and 27 and found that the former, which attacks a greater variety of hosts, had a much wider range of maximum germination with respect to hydrogen ion concentration, than had form 27. The investigations of form 21, which compares with form 11 in its host range, show that it has a still more restricted range of maximum germination than Hursh reported for form 27. It appears therefore that Hursh's hypothesis is unsound.

Effect of Phenolic Compounds on the Germination of Urediniospores of Form 21

Materials and Methods

Rust spores were cultured by the method described previously. The compounds used were pure. Most of them were obtained from the Eastman Kodak Company, others had been prepared in the laboratory.

Since hydrogen ion concentration has such a marked effect on the germination of the rust spores, and since solutions of the various compounds used differ in their hydrogen ion concentration, both buffered and unbuffered solutions of the compounds were studied. Solutions of the compounds and of Clark and Lubs' hydrochloric acid and phthalate buffer solution for pH 6.0 were made up at double the required concentration and equal quantities of each were mixed together.

Germination tests were carried out as described previously. The approximate concentration at which each compound prevented germination was determined by the method of trial and error. A number of concentrations about this point were then tested in an attempt to determine more accurately the exact concentration which would just prevent germination. The number of compounds which could be tested and the highest concentration at which they could be used were limited by the solubility of the compounds in water.

Experimental Results

A summary of the results is reported in Table III. For the more toxic and more soluble compounds, the concentration at which germination was completely inhibited is reported. In other cases the percentage germination is reported for the highest concentration tested.

TABLE III

EFFECT OF PHENOLIC COMPOUNDS ON GERMINATION

—	Buffered (pH 6)		Unbuffered		
	Conc., p.p.m.	*Germ.*, %	*Conc.*, p.p.m.	*Germ.*, %	pH
Phenols					
Hydroquinone	45	0	15	0	5.7
Catechol	340	0	200	0	4.6
Guaiacol	400	0	—	—	—
Phenol	600	0	600	0	7.1
Pyrogallol	800	4	1600	0	6.2
Resorcinol	750	6	1200	0	5.1
Phloroglucinol	800	55	1600	47	5.0
o-Cresol	125	0			
m-Cresol	125	61			
Phloracetophenone	200	22			
Quercetin	400	34			
Quercitrin	400	34			
Phenolic acids, etc.					
Anisic acid	180	0	40	0	4.2
Benzoic acid	250	0	25	0	—
Salicylic acid	800	4	60	0	3.6
Protocatechuic acid	800	16	400	0	3.6
o-Methoxybenzoic acid	800	16	200	0	3.6
Vanillic acid	800	38	600	0	3.3
p-Methoxybenzoic acid	800	58	800	0	—
Gallic acid	800	68	800	3	3.4
β-Resorcylic acid	800	72	800	2	—
Aconitic acid	800	72	800	2	2.8
Syringic acid	800	88	800	0	3.3

DISCUSSION

The data reported must be considered as approximate, though they are probably sufficiently accurate for the purpose of placing the compounds in the order of their toxicity. The exact point at which each compound prevents germination is not readily determined. With some compounds germination falls off steadily as the concentration is increased, but with others the germination falls to about 5 to 10% and then decreases very slowly as the concentration is increased. In the latter cases the concentration at which germination is entirely inhibited is difficult to determine and may not adequately represent the toxicity of the compound.

It appears that if this investigation is extended to include the study of the effects of a number of compounds on different rust forms, the method used in the work described in this paper would not be suitable. Repetition of a series of simultaneous determinations of the effects of a number of different concentrations of different compounds on the various rust forms and statistical analyses of the data would probably yield more accurate results.

The inhibitory effect of the compounds, of which the bactericidal properties have already been studied (5, and papers cited therein), on the germination

of urediniospores of form 21, is fairly closely related to their bactericidal properties. Because of the small number of compounds of the same category tested, no attempt can be made to offer a hypothesis relating chemical constitution to inhibitory effect. The possible importance of the positions of two similar groups in the nucleus is amply illustrated by the differences in toxicity between *o*- and *m*-cresol and between catechol, resorcinol and hydroquinone. In this connection, however, it must be pointed out that physical properties may be more important than chemical ones in determining the toxicity of the compounds.

During the course of these studies it was observed that concentrations of certain of the compounds, of such strength that germination was scarcely affected, stimulated the growth of the germ tubes. This effect was particularly marked with a solution of 200 p.p.m. of phenol in which the germ tubes attained a much greater length than those of spores germinated in water. This observation is in accord with our knowledge of the action of poisons, most of which are stimulative if taken in small enough doses. The observation is also of interest in connection with the results of comparisons of the effects of host extracts made by estimating the inhibitory effect on spore germination and on the growth of germ tubes; it is discussed further in a later section of this paper.

Effect of Host Extracts on the Germination and Growth of Germ Tubes of Urediniospores of Form 21

Materials and Methods

Four wheat varieties were used in these studies, namely, Vernal, Khapli, Marquis and Little Club. The first two varieties are resistant to form 21 and the last two are susceptible. Stakman and Levine (9) record the reactions as follows:

Variety	Range	Mean
Vernal	0 to 1	0 ;
Khapli	0 to 1	1 =
Marquis	3++ to 4+	4
Little Club	3++ to 4++	4

The plants were grown in the greenhouse. Separate seedings were made for each series of determinations. The plants were harvested ten days after emergence by cutting them off just above the ground level. The press-juice was obtained by the method of Newton, Brown and Martin (6). It was centrifuged, decanted and diluted with distilled water to the required concentration. Harvesting and all subsequent operations were carried out for all four varieties as nearly concurrently as possible.

The press-juice of each variety was tested at four concentrations, namely, 15, 10, 5 and 2.5%. Four dishes were used for each concentration of each

variety and 250 spores were counted in each dish. Seeding, incubation, and counting of the spores were carried out in the manner previously described.

In planning the investigation no provision was made for measuring the length of the germ tubes, since to do this on a scale commensurate with the determinations of percentage germination would have required more time than was available. However, during the course of the investigations rough and rapid estimates of the length of the germ tubes were made. The mean length of the tubes in each dish was estimated by selecting two or three tubes, which appeared by inspection to be of average length, and measuring and averaging these. The method is open to serious objections from the statistical point of view and no great reliance can be placed upon the results.

The total solids of the undiluted press-juice were determined by means of the refractometer, and the hydrogen ion concentrations of the 15 and 2.5% dilutions were determined potentiometrically.

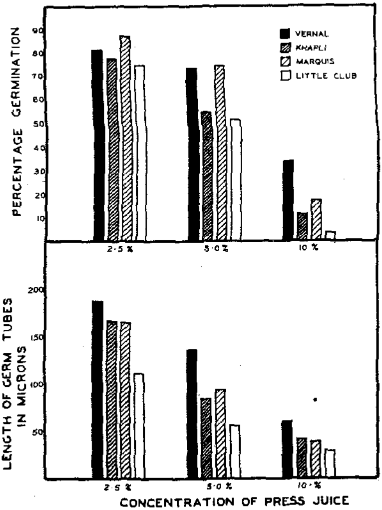

FIG. 2. *Effect of host extracts on (upper) percentage germination and (lower) growth of germ tubes of urediniospores of form 21.*

Experimental Results

The results of ten series of determinations are given in Table IV. The values under percentage germination represent the mean of four counts of 250 spores; the values under length of germ tubes represent the mean of estimates of length made for each of four dishes. In order to facilitate examination of the data, they are given in condensed form in Table V as the mean for all varieties by series, and in Table VI as the mean for all series by varieties. The data on the percentage germination and on the length of germ tubes given in Table VI are also presented graphically in Fig. 2.

TABLE IV

EFFECT OF HOST EXTRACTS IN CONCENTRATIONS OF 15, 10, 5 AND 2.5% ON THE GERMINATION OF SPORES AND ON THE GROWTH OF GERM TUBES

Date, 1933	Variety	Total solids	pH		Mean percentage germination					Mean length, microns			
			15%	2.5%	15%	10%	5%	2.5%	0%	10%	5%	2.5%	0%
June 9	Vernal	6.2	6.23	6.29	0.2	4.1	55.8	67.1	87.0	—	98	153	330[1]
	Khapli	5.4	6.03	6.12	0.4	3.3	41.0	70.9		—	65	140	
	Marquis	6.2	6.22	6.26	0.3	23.0	62.6	84.6		—	88	153	
	L. Club	7.1	6.03	6.12	0.2	1.6	44.2	64.4		—	42	91	
June 13	Vernal	6.2	6.45	6.43	19.3	40.2	78.9	64.5	91.9	88	225	195	
	Khapli	5.8	6.34	6.32	0.7	23.5	72.5	76.2		39	143	192	
	Marquis	6.2	6.35	6.33	0.1	16.3	82.7	89.4		33	91	182	
	L. Club	6.6	6.36	6.33	0.1	5.3	53.9	84.4		26	46	130	
June 16	Vernal	6.2	6.29	6.37	18.9	66.1	89.2	92.5	87.7	96	183	225	
	Khapli	5.8	6.15	6.32	0.9	22.5	65.9	89.3		53	125	198	
	Marquis	5.4	6.30	6.33	0.6	24.9	83.6	87.7		38	85	171	
	L. Club	6.2	6.18	6.24	0.0	5.6	70.8	85.3		30	60	133	
June 20	Vernal	5.8	—	6.18	2.1	45.0	76.5	88.2	92.7	49	137	205	
	Khapli	6.2	—	6.16	0.2	5.9	67.6	87.4		39	55	150	
	Marquis	7.5	—	6.06	0.9	36.7	88.3	90.9		42	114	221	
	L. Club	6.2	—	6.15	0.0	1.4	72.8	89.2		—	65	101	
June 23	Vernal	5.8	6.50	6.65	0.0	1.9	72.5	92.8	92.0	—	65	173	
	Khapli	5.0	6.32	6.40	0.0	8.3	62.6	92.2		33	73	183	
	Marquis	6.2	6.26	6.37	0.2	9.7	74.9	95.5		38	68	145	
	L. Club	6.6	6.22	6.35	0.1	0.2	56.0	75.2		—	45	100	
June 27	Vernal	7.1	6.33	6.40	5.1	43.9	74.1	81.5	87.7	43	140	190	
	Khapli	5.0	6.15	6.27	0.2	10.7	51.3	76.9		35	90	160	
	Marquis	5.8	6.28	6.30	0.6	16.4	71.8	87.3		40	80	135	
	L. Club	6.6	6.18	6.34	0.0	2.6	38.7	67.8		30	40	125	
June 30	Vernal	5.4	6.20	6.30	19.6	46.3	58.5	80.6	94.6	50	120	245	
	Khapli	5.0	6.03	6.10	0.0	2.6	49.1	63.3		30	73	143	
	Marquis	5.4	6.30	6.30	0.0	6.1	59.1	77.5		30	65	153	
	L. Club	5.8	6.20	6.30	0.0	1.5	35.5	64.6		—	38	80	
July 4	Vernal	7.1	6.17	6.34	10.1	34.6	82.7	89.6	94.3	40	98	143	
	Khapli	5.8	6.00	6.05	7.0	10.3	57.6	88.1		38	78	178	
	Marquis	6.2	6.20	6.38	0.0	0.6	61.2	89.1		—	60	108	
	L. Club	—	6.00	6.09	0.0	0.5	40.0	87.0		—	60	110	
July 7	Vernal	7.1	6.20	6.40	0.8	19.4	65.7	73.0	91.8	30	138	175	
	Khapli	5.8	6.06	6.20	0.0	0.7	39.3	60.9		—	60	133	
	Marquis	5.4	6.00	6.30	0.2	12.4	79.1	85.8		—	125	185	
	L. Club	—	6.08	6.14	0.0	16.4	70.5	75.3		30	105	125	
July 12	Vernal	6.2	6.18	6.35	34.4	38.6	79.8	82.7	88.6	83	153	185	
	Khapli	5.4	6.00	6.32	2.0	26.2	35.7	71.8		65	88	195	
	Marquis	5.8	6.06	6.24	4.6	25.6	79.7	85.6		55	160	200	
	L. Club	7.5	6.06	6.30	0.0	1.7	30.1	52.6		—	63	128	

[1] *Average length of germ tubes of spores sown on water.*

TABLE V

EFFECT OF HOST EXTRACTS ON THE GERMINATION OF SPORES AND THE GROWTH OF GERM TUBES

(Means by days)

Date	Total solids	pH	Percentage germination					Length in microns			
			15%	10%	5%	2.5%	0%	10%	5%	2.5%	0%
June 9	6.2	6.17	0.3	8.0	50.9	71.8	87.0	—	73	134	330
13	6.2	6.37	5.1	21.3	72.0	78.6	91.9	47	126	175	
16	5.9	6.28	5.1	29.8	77.4	88.7	87.7	54	113	182	
20	6.4	6.14	0.8	22.3	76.3	88.9	92.7	(43)	93	169	
23	5.9	6.39	0.1	5.0	66.5	88.9	92.0	(36)	63	150	
27	6.1	6.29	1.5	18.4	59.0	78.4	87.7	37	88	153	
30	5.4	6.22	4.9	14.1	50.6	71.5	94.6	(37)	74	155	
July 4	6.4	6.16	4.3	11.5	60.4	88.5	94.3	(39)	74	135	
7	6.1	6.18	0.3	12.2	63.7	73.8	91.8	(30)	107	155	
12	6.2	6.19	10.3	23.0	56.3	73.2	88.6	(68)	116	177	

Brackets indicate mean of less than four varieties.

TABLE VI

EFFECT OF HOST EXTRACTS ON THE GERMINATION OF SPORES AND THE GROWTH OF GERM TUBES

(Means by varieties)

Variety	Total solids	*pH*	Percentage germination					Length in microns			
			15%	10%	5%	2.5%	0%	10%	5%	2.5%	0%
Vernal	6.3	6.33	11.0	34.0	73.4	81.3	90.8	60	136	189	330
Khapli	5.5	6.18	1.1	11.4	54.3	77.7		42	85	167	
Marquis	6.0	6.25	0.8	17.2	74.3	87.3		39	94	165	
L. Club	6.6	6.20	0.0	3.7	51.3	74.6		29	56	112	

STATISTICAL ANALYSES

Analyses of Variance of Percentage Germination and Length of Germ Tubes

The data on the percentage germination and the length of germ tubes were subjected to an analysis of variance. The data obtained by each method of estimating inhibitory effect were analyzed separately, and in both cases the results at each concentration were also examined separately. The variance of the observed results was analyzed into portions due to; (i) variations in the general level of the results obtained in different series, (ii) to the average differences, over all series, between varieties, (iii) to differences in the relative performance of the varieties in different series, and (iv) to differences between parallel counts. The results of these analyses are summarized in Table VII. No analysis is reported for the length of germ tubes in 10% concentrations of the press-juice as the data for these determinations were incomplete.

TABLE VII

ANALYSES OF VARIANCE OF GERMINATION AND LENGTH OF GERM TUBE

Variance due to	Degrees of freedom	Mean squares					
		Percentage germination				Length of germ tubes	
		15%	10%	5%	2.5%	5%	2.5%
Series	9	1074	5906	9393**	5960**	7640*	3848
Varieties	3	6821**	41450**	37443**	7493**	42848**	42334**
Series × Varieties	27	812††	3123††	2187†	1309††	3118††	2762††
Parallels	120	226	767	1176	539	816	1235

* *and* ** *Significantly greater than the mean square due to series × varieties and to that due to parallels.*

† *and* †† *Significantly greater than the mean square due to parallels.*

The analyses of variance show by the magnitude of the mean squares due to series and to the interaction between series and varieties, that there was considerable variation in the results from series to series. It is presumed that the main factors contributing to this are the variations in spores and wheat plants, and possible slight differences in technique.

The mean square due to series, since it represents that portion of the variation between series which affects all varieties to the same extent, may be eliminated from the comparisons of the data. That portion of the variation between series which affects the varieties differentially, that is, the interaction between series and varieties, is of considerable importance. The analyses show that the mean square due to the interaction was in all cases significantly greater than the mean square due to variance in the parallel counts. It is therefore the variance due to the interaction and not that due to parallel counts which is the main factor in reducing the accuracy of the comparisons of varieties.

Two factors which probably play a part in producing the differences in the relative performances of different varieties in different series suggest themselves: first, the variations in the total solids of the press-juice both within and between varieties over the ten series; and, second, similar variations in the hydrogen ion concentration of the press-juice. Investigations of the possible effects of these factors are described later.

Notwithstanding the comparative magnitude of the interaction effect, the main object of this investigation, the establishment of significant differences between varieties, was attained, as is shown by the fact that the mean square due to varieties is in all cases significantly greater than those due to the interaction and parallel counts. Or, in other words, in spite of the different relative performances of the varieties in different series, the average performances of the varieties, over all series, differed significantly. In order to facilitate the comparison of the varieties, the differences between the means for all series, for each pair of varieties, are given in Table VIII. The smallest differences

between the means, required for a 5% level of significance, were calculated from the mean squares due to the interaction by the method previously described. The values obtained are also given in Table VIII.

TABLE VIII

DIFFERENCES BETWEEN VARIETAL MEANS FOR ALL SERIES

Difference	Percentage germination				Length of tubes	
	15%	10%	5%	2.5%	5%	2.5%
L. Club – Marquis	– 0.8	**–13.5**	**–23.0**	**–12.7**	**–38**	**–53**
L. Club – Khapli	– 1.1	– 7.7	– 3.0	– 3.1	**–29**	**–55**
L. Club – Vernal	**–11.0**	**–30.3**	**–22.1**	**– 6.7**	**–80**	**–77**
Marquis – Khapli	– 0.3	+ 5.8	**+20.0**	**+ 9.6**	+ 9	– 2
Marquis – Vernal	**–10.2**	**–16.8**	+ 0.9	+ 6.0	**–42**	**–24**
Khapli – Vernal	**– 9.9**	**–22.6**	**–19.1**	– 3.6	**–51**	–22
Necessary difference for 5% level	5.2	10.3	8.6	6.6	26	24

Numbers in bold face are significant differences.

The differences were obtained by subtracting the value for the more resistant variety from that for the less resistant. A positive difference therefore indicates that the extract of the more resistant variety had a greater inhibitory effect, and supports the hypothesis that the inhibitory effect of host extracts is directly correlated with the rust resistance of the hosts. It will be observed that of the 36 differences reported in Table VIII, only 6 are positive; and that of the 23 differences which are significant, only 2 are positive. Further, if the effect of host extracts on spore germination or the growth of germ tubes had any relation to the rust resistance of the host, it seems reasonable to suppose that the varieties would have been divided into two groups; the resistant varieties, Vernal and Khapli, and the susceptible varieties, Marquis and Little Club, being paired. The results show no such pairing.

Investigation of the Possible Effect of Variations in Total Solids

The variance of the total solids of the press-juice was also analyzed; the results are given in Table IX. The mean square due to the varieties was not significantly greater than that due to the remainder. In other words, the relative quantities of total solids in the press-juice from the four varieties differed so much between series that it cannot be said that the means for the varieties, for all series, differ from each other significantly. In these circumstances it is apparent that the differences in total solids of the press-juice could have had no consistent effect upon the results of the main investigation.

TABLE IX

ANALYSIS OF VARIANCE OF TOTAL SOLIDS OF THE PRESS-JUICE

Variance due to	Degrees of freedom	Sum of squares	Mean square
Varieties	3	3.654	1.218
Series	9	4.632	.514
Remainder	27	17.742	.630

Investigation of the Possible Effect of Variations in Hydrogen Ion Concentration

The data for the hydrogen ion concentration of 2.5% concentrations of the press-juice were also subjected to an analysis of variance; the results are given in Table X.

The mean square due to the varieties proved to be significantly higher than that due to the remainder. The necessary difference between the means for varieties, for all series, calculated for a 5% level of significance, was found to be 0.05 pH units. The press-juice of Vernal has the lowest hydrogen ion concentration, pH 6.37, followed by that of Marquis, pH 6.29, with those of Khapli and Little Club, pH 6.23 and 6.24, highest.

TABLE X

ANALYSIS OF VARIANCE OF HYDROGEN ION CONCENTRATION OF THE PRESS-JUICE

Variance due to	Degrees of freedom	Sum of squares	Mean square
Varieties	3	0.1318	0.0439††
Series	9	.2732	.0304
Remainder	27	.1476	.0051

†† *Significantly greater than the mean square due to remainder.*

Reference to the first part of this paper will show that the data obtained on the effect of hydrogen ion concentration on spore germination indicate that if the concentration at which maximum germination takes place could be determined with precision it would be found to lie in the neighborhood of pH 6.2. If hydrogen ion concentration had any considerable effect on the germination of spores in 2.5% concentrations of the host extracts, it would appear that Vernal and Marquis should have shown a greater inhibitory effect than Khapli and Little Club. The results show, on the contrary, that the reverse was true, thus indicating that hydrogen ion concentration was not an important factor.

In order to investigate further the possibilities of a significant correlation between percentage germination and hydrogen ion concentration, the data obtained with 2.5% concentrations of the press-juice were subjected to an analysis of variance and covariance. The relationship was assumed to be curvilinear and the percentage germination was correlated with both the pH and the square of the pH. The coefficients of regression of percentage germination on pH and on the square of the pH were calculated and their significance tested by determining the proportion of the total variance in germination capable of representation by the regression equation. The mean square due to the regression did not differ significantly from the mean square deviation, showing that there was no appreciable correlation between percentage germination and hydrogen ion concentration. It is concluded, therefore, that the variations in hydrogen ion concentration within and between varieties, and between series, had no consistent and significant effect upon the results of the main investigation.

In this connection, however, it is interesting to note that the results of a single series of determinations, carried out as an afterthought, in which the

press-juice was diluted with buffer solutions of pH 6.1, instead of with distilled water, did not agree entirely with the results of the main series of investigations. The data obtained are given in Table XI.

TABLE XI

THE EFFECT OF HOST EXTRACTS DILUTED WITH A BUFFERED SOLUTION ON THE GERMINATION AND GROWTH OF GERM TUBES OF UREDINIOSPORES

Variety	Mean percentage germination					Mean length, microns		
	15%	10%	5%	2.5%	0%	10%	5%	2.5%
Vernal	14.2	21.5	21.1	28.8	89.1	54	53	72
Khapli	3.1	7.5	28.2	32.5	—	19	71	81
Marquis	9.5	19.2	41.1	56.3	—	48	103	141
L. Club	0	5.8	36.4	35.5	—	15	91	89

It will be observed that at the lower concentrations, the varieties fall in a more logical order. That it would be unwise to place much weight upon the results of a single series is amply illustrated by the fact that the order of the varieties, as judged by the effect of 2.5% concentrations on the germination of spores, is exactly the same in the series carried out on June 13, diluting with water, as it is in the series under discussion. Unfortunately, owing to the incidence of hot weather and consequent irregularity in the germination of the spores, it was not possible to carry out further investigations of the effects of press-juice diluted with buffered solutions. The results of the single series involving this technique are included since they suggest possibilities for further investigations.

Relation between Percentage Germination and Length of Germ Tubes

The correlation coefficients between percentage germination and length of germ tubes for each variety were calculated and found to be as follows: Vernal 0.79, Khapli 0.88, Marquis 0.80, and Little Club 0.80. The scatter diagrams for the four varieties are shown in Fig. 3. The numerals represent the number of the series. Inspection of the diagrams will show that the correlations are not biased by differences in the general level of the results from series to series. There is, therefore, a high degree of correlation within varieties, between the two methods of estimating inhibitory effect.

The fact that the varieties did not fall in exactly the same order at different concentrations, when placed according to the effect of extracts on germination and on the growth of the germ tubes, suggests that the relation between percentage germination and length of germ tubes is not the same for all varieties. The scatter diagrams offer evidence in support of this conclusion. It will be observed that the slope of the regression line is not the same in all four diagrams. The numerical values for the slope are as follows: Vernal 2.51, Khapli 1.79, Marquis 1.59, and Little Club 1.11. In addition it will be observed that the relationship in the case of Marquis appears to be definitely

curvilinear whereas those for the other three varieties are almost linear. In these circumstances, it is not surprising that the two methods of comparing the inhibitory effect of extracts of different varieties did not yield exactly the same results at different concentrations.

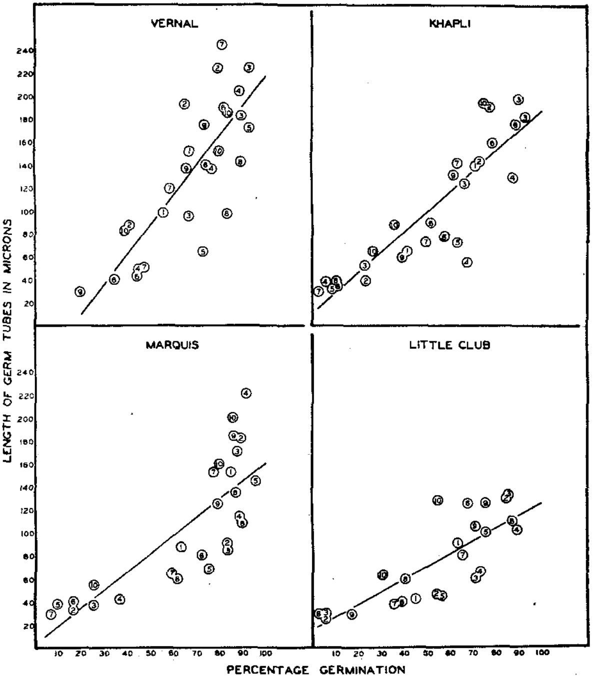

FIG. 3. *Correlation between the effects of host extracts on the germination of urediniospores of form 21 and on the growth of germ tubes.*

Discussion

The results of the investigation of the effects of host extracts on the germination of urediniospores and the growth of germ tubes are characterized by extreme variability. In these circumstances the interpretation of the results, even with the aid of statistical methods, is not easy. The statistical treatment

serves to divide the variance into a number of portions, *viz.*: a portion due to differences in parallel counts; a portion due to differences between varieties; a portion due to differences between series which affect all varieties to an equal extent; and a portion due to differences between series which affect the varieties differentially. The results also show that variations in concentration of press-juice exert a differential effect not only on different varieties but also on the germination and growth of germ tubes of the same variety.

It seems safe to assume that the variability in parallel counts is largely the result of sampling errors. These arise in several ways. The limiting error, in all probability, is the fact that the 250 spores counted, and more particularly the two or three germ tubes selected for measurement, do not constitute a representative sample. Additional errors may be introduced by the fact that a representative sample of the original spores may not be transferred to each dish and by the fact that the distribution of the spores in the dish also affects germination and growth. The results show, however, that the variability of the parallel counts was of comparatively little importance since it was by no means the limiting error of the determinations as a whole.

That part of the variation between series which affects all varieties to the same extent, though of considerable magnitude, is of little importance from the point of view of comparing varieties. These variations are probably attributable to differences in the conditions of the spores and wheat plants used in different series, presumably caused by variations in the environmental conditions in the greenhouse.

That part of the variation between series which affected the varieties differentially constituted the limiting error of the determinations. The results indicate quite definitely that the relative inhibitory properties of the extracts of different varieties differed from day to day. Since the plants of each variety, for any given series, were grown under conditions as nearly similar as possible, it seems reasonable to suppose that differences in the environmental conditions under which the plants of different series were grown affected their constitutions differentially, and thus had differential effects upon the inhibitory properties of their extracts. Evidence supporting this conclusion is offered by the fluctuation from series to series in the relative quantities of total solids in the press-juice of the different varieties, and by similar variations in hydrogen ion concentration.

The fact that variations in total solids of the press-juice had no consistent effect upon the results of the investigation is difficult to explain. It seems probable that the quantities of the various compounds in the press-juice varied independently and that measurement of total solids was therefore not a measure of the quantity of the inhibitive compound or compounds present in the extracts. It is also possible that physical properties not reflected by an estimate of total solids are important factors.

Differences in the relative inhibitory effects of the varietal extracts, at different concentrations, and on germination and growth, are not as important as might be thought at first glance. It is true that the order of the

varieties with respect to inhibitory effect differs for different concentrations, but reference to Table VIII will show that in no case was a variety shown to have a significantly greater inhibitory effect than another variety at one concentration and a significantly less effect at another. Neither did the results of one method of measuring inhibitory effect contradict those obtained by the other. The cases in which the order of the varieties is changed from one concentration to another, or between methods, are those in which the methods failed to differentiate the varieties.

In spite of the variations discussed above, both tests were sufficiently accurate to show that differences exist between the average inhibitory effects of extracts of certain of the varieties. When placed in order of increasing inhibitory effect on germination, the varieties fall in the following order: Vernal, Marquis, Khapli and Little Club. The germination test fails to separate Vernal and Marquis at the two lower concentrations, Marquis and Khapli at the two higher concentrations and Little Club and Khapli at all concentrations. When placed in order of increasing inhibitory effect on the growth of germ tubes, the varieties fall in the same order. This method fails to differentiate Marquis and Khapli. The results as a whole fail to show any relation between the effect of host extracts on the germination of urediniospores, or on the growth of germ tubes, and the rust resistance of the variety.

These conclusions do not agree with those of Ezekiel. On the other hand, they do not disprove his hypothesis. Ezekiel worked with such dilute extracts of the hosts that the growth of the germ tubes was increased over that of the controls. In the investigations reported in this paper, more concentrated extracts were used and the growth of the germ tubes was inhibited in all experiments with such extracts.

In the course of the investigation of the effects of phenol on the germination of urediniospores it was noted that a solution of 200 p.p.m., which had a comparatively slight effect on germination, stimulated the growth of the germ tubes remarkably. At increased concentrations the germination of the spores was reduced and the growth of the tubes inhibited. At a concentration of 600 p.p.m. no germination or growth took place. Ezekiel's results, together with those reported in this paper, suggest that host extracts behave similarly; that although they reduce growth at high concentrations they stimulate it at very low concentrations. However, there appear to be no adequate reasons for assuming that different varieties would fall in the same order when placed according to the inhibitory effects of their concentrated extracts on rust spores, and according to the stimulating effects of very dilute extracts.

Acknowledgments

The author is indebted to Mr. E. K. Woodford, undergraduate research assistant, Department of Field Crops, University of Alberta, for the experimental work involved in these investigations, and to Dr. J. W. Hopkins of the Division of Biology, National Research Laboratories, Ottawa, for advice on the statistical treatment of the data.

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THE EFFECT OF POTASSIUM SUPPLY ON THE COMPOSITION AND QUALITY OF WHEAT[1]

By A. G. McCalla[2]

Abstract

The amount of minerals absorbed by wheat plants and the time of absorption influenced the quality of the grain produced. The quality was adversely affected by restriction of potassium supply, and this effect could not be overcome by supplying sodium in place of potassium. The quality of the grain produced by plants which had a small supply of potassium from germination until heading was better than that of grain from plants which absorbed the same amount of potassium in the first month of growth, but none from then on.

Introduction

In 1907, Wood (17) suggested that the acids and salts in the cell sap of wheat when the endosperm is forming impress upon the gluten the physical properties which decide the character of the flour obtainable from that wheat. This suggestion, in modified form, has been revived by Gericke (4, 5), who advanced the thesis that the variation in the quality of protein of wheat flour, as determined by baking methods, can be partially accounted for by colloidal phenomena expressing themselves in the physical properties of proteins and starches, the nature of these physical properties being determined by the absorption of certain inorganic elements in excess of the amounts required for the production of the crop. Experimentally, Gericke (4) found that better bread was obtained from wheats which had a relatively large excess of salts in the straw, beyond the minimum required for the yield obtained, than from those which had no excess or only a slight one.

More recently, Gericke (6) studied the effect of supplying maturing wheat plants with nitrate in combination with various cations. He demonstrated that the quality of the bread produced from these wheats varied with the nature of the cation of the salt used to supply the nitrogen, and concluded that the mineral nutrition of the wheat plants markedly affects the quality of the protein produced in the grain.

As the baking quality of flour is dependent to a large extent on the quality of the gluten laid down in the wheat grain, then, on the basis of Gericke's results, variations in the nutrition of the wheat plant will of necessity have an influence on the quality of the flour. In an earlier paper (11) the writer reported a detailed study of the effect of limiting the nitrogen supply on the composition and quality of wheat. The general conclusion reached was that, although the course of nitrogen metabolism in the vegetative parts of the plant was altered, and the total nitrogen content of the grain markedly influenced, the quality of the gluten produced in the wheat grain was not affected by limiting the supply of inorganic nitrogen.

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Probably the availability of many elements and the physiological relations existing among them play important parts in determining the course of nitrogen metabolism. A survey of the literature reveals that potassium is considered essential in the early stages of this process, but there is far from unanimous agreement regarding the most important role of this element in the development of green plants. Hoagland (8) points out that researches on potassium functions are necessarily concerned with resultant effects of potassium deficiency on growth and metabolism. Although it is difficult to allocate the functions of potassium to specific steps in the whole chain of metabolic processes, recent literature indicates that increasing attention is being paid to nitrogen metabolism and cell division.

Ginsburg (7) studied the effect of omitting potassium from culture solutions in which soy beans were growing. He found that the total nitrogen content of the plants grown in a solution in which the lack of potassium was compensated for by calcium, was less than half, but the calcium content more than double, that of the check plants. When the lack of potassium was compensated for by sodium, the amount of total nitrogen absorbed was greater, and the amount of calcium slightly less, than where calcium was used.

Potassium-deficient soy beans were also studied by Burrell (2), who concluded that one important role of potassium was in the formation of proteins from carbohydrates and amino acids. This conclusion was based on the fact that the amount of insoluble nitrogen was less, but that of the non-protein fraction, particularly amino nitrogen, greater in potassium-deficient than in normal plants.

Burrell's conclusion was supported by the work of Nightingale *et al* (13) with potassium-deficient tomato plants. They concluded that potassium was probably essential for the synthesis of proteins in meristematic tissues, but probably not essential for later stages of storage protein synthesis.

These investigators also found that nitrate assimilation was retarded in potassium-deficient plants, and concluded that potassium was directly or indirectly essential in the initial stages of nitrate reduction. In the same publication, it is reported that Dr. Eckerson found that the nitrate reducing power of potassium-deficient beet and lettuce plants was much less than that of the check plants.

Loew (10) discussed the role of potassium in green plants and attributed its specific activity to the fact that potassium compounds present in protoplasm emit β-rays and give rise to physiological oxidation processes, thus promoting the generation of energy in the living cell. He concluded that it must necessarily follow that potassium is concerned with the building up of protein bodies in plant cells.

All of the authors cited so far agree that potassium promotes the production of higher nitrogen compounds in green plants, but Janssen and Bartholomew (9), working with cowpeas and sugar beets, found that potassium-deficient plants contained higher percentages of total nitrogen than did the check

plants. They also found that the percentage of protein nitrogen was higher in the deficient plants. The same authors (1) found that very low concentrations of potassium maintained in the culture solution sufficed for the needs of the plants, and that the potassium absorbed during the early stages of growth could be translocated and re-utilized.

There is not, as far as the writer knows, any record of culture studies carried out for the specific purpose of determining the effect of marked potassium deficiency on the quality of the grain produced by cereals. Gericke (6) has shown that the absorption of potassium nitrate by wheat plants after the time of heading is beneficial to the quality of the grain. There is evidence from various field experiments, such as that carried out by Scharnagel (15), that fertilizing wheat with potassium salts improves the quality, although, in some instances at least, it may actually lower the nitrogen content of the grain.

It seems probable that the various effects of limited potassium supply as noted in the preceding paragraphs would have an influence on the quality of wheat produced in water cultures. The present study deals with preliminary experiments on the effect of limited potassium supply on the composition and quality of wheat.

Experimental

Production of Material

The material used in this study was Marquis wheat grown in culture solutions during the spring and summer of 1933. Five lots of plants were produced, the equipment and methods described in an earlier paper (11) being used. The culture solutions supplied to these lots during the various stages of development are recorded in Table I, and the chemical composition of each culture solution in Table II.

TABLE I

Solutions supplied to the various cultures during three periods of growth

Culture No.	First month	To heading	To maturity	Abbreviated description
1	Complete	Complete	Complete	Complete
2	Complete	Complete	Tap water	Complete: water
3	Low K, with Na	Low K, with Na	Tap water	Low K, Na: water
4	Low K, with Ca	Low K, with Ca	Tap water	Low K, Ca: water
5	Complete	K-free with Ca	Tap water	Complete: K-free: water

At the end of the first month the tanks were all drained and refilled with the same solutions except that a potassium-free solution was supplied to culture 5. The small amount of potassium supplied to the low potassium cultures 3 and 4 during the first month was continued during the second.

The total amount of potassium supplied to these cultures over the whole period of development was 11.7 mgm. in the form of 0.30 cc. of molar potassium dihydrogen phosphate per litre of solution.

Ferric tartrate was added to each culture every third day for the first month, and then every second day until vegetative growth ceased.

TABLE II

CHEMICAL COMPOSITION OF CULTURE SOLUTIONS EXPRESSED AS CC. OF MOLAR SALT SOLUTION PER LITRE OF CULTURE SOLUTION

Solution	KNO_3	$Ca(NO_3)_2$	$NaNO_3$	KH_2PO_4	$Ca(H_2PO_4)_2$	NaH_2PO_4	$MgSO_4$	Ferric tartrate
Complete	5	5	—	1	—	—	2	1
Low K with Na	—	5	5	0.15*	—	0.85	2	1
Low K with Ca	—	7.5	—	0.15*	0.85**	—	2	1
K-free with Ca	—	7.5	—	—	0.85**	—	2	1
Tap water †	—	—	—	—	—	—	—	1

* *Added as 0.05 cc. once every 10 days for 2 months.*

** *Added as 6.8 cc. of* $\frac{M}{16}$ $Ca(H_2PO_4)_2$.

† *Tap water contained about 200 p.p.m. of salts but no N or K.*

When the plants were mature, the samples were collected and threshed. The total nitrogen, ash, potassium, calcium, magnesium, phosphate and sulphate contents of straw (including chaff) and grain were determined. The nitrogen compounds of the grain were studied in some detail.

METHODS OF ANALYSIS

Total nitrogen and nitrogen fractions. The methods used for determining total nitrogen and the various nitrogen fractions have been discussed in an earlier paper (11). In addition, however, arginine nitrogen in the gluten hydrolysate was determined by the procedure outlined by Plimmer and Rosedale (14).

Ash. Approximately two-gram samples of ground straw and five-gram samples of grain were ashed in a muffle furnace at 570° C. for six hours. The ash samples were taken up in dilute hydrochloric acid, and the solutions made up to 100 cc. Aliquots were used in the determination of the mineral elements.

Potassium. Potassium was determined on 5 cc. aliquots of the ash solution using the colorimetric method described by Morris and Gerdel (12).

Calcium. Calcium in 40 cc. aliquots of the ash solution was precipitated as calcium oxalate and the oxalate titrated with $N/20$ potassium permanganate.

Magnesium. Magnesium in the filtrate from the calcium determination was precipitated as magnesium ammonium phosphate, taken up with $N/14$ sulphuric acid, and the excess acid titrated with $N/14$ sodium hydroxide.

Phosphate. Phosphate was determined on 2-cc. aliquots of the ash solution using the colorimetric method as described by Truog and Meyer (16).

Sulphate. Sulphate in 50-cc. aliquots was precipitated with barium chloride, ignited and weighed as barium sulphate.

Results

The results are summarized in Tables III to XI. The discussion of the tabulated data is largely restricted to the differences resulting from potassium nutrition.

Grade and Weight of Grain

The grade, weights per bushel and weight per 1000 kernels of each of the five samples are presented in Table III. The grain from complete culture plants was de-graded because the kernels were somewhat shrivelled, although they were hard and vitreous. The cause of this shrivelling has been discussed in an earlier paper (11). It was concluded that the synthesis of organic nitrogen compounds from nitrates, which continued for some time after vegetative growth and the production of carbohydrates had ceased, used considerable quantities of soluble carbohydrates which would otherwise have been translocated to the grain as such. The removal of these carbohydrates in the vegetative parts of the plant resulted in the incomplete filling of the developing kernels, and the consequent shrivelled appearance. None of the results in the present study have given any indication that these conclusions need be altered. The low potassium, calcium: water, and complete: potassium-free: water samples were de-graded because they contained some starchy kernels.

TABLE III

Weight and grade of mature kernels

Culture No.	Description of culture	Time to maturity,* days	Grade	Weight per bushel, lb.	Weight per 1000 kernels, gm.	Grain as % of total weight of crop
1	Complete	113	2 Northern	63	33.51	33.8
2	Complete: water	106	1 Hard	65	28.64	37.0
3	Low K, Na: water	106	1 Hard	63	22.43	36.6
4	Low K, Ca: water	106	2 Northern	62.5	26.09	38.7
5	Complete: K-free: water	106	1 Northern	63.5	26.32	38.9

* *Time taken from first soaking of seeds.*

Table III shows that the weight per bushel was greatest in the grain from No. 2 culture, with probably insignificant differences among the weights of the grain from other cultures. It is obvious that weight per bushel is not related to weight per 1000 kernels, nor is weight per 1000 kernels related to grade. All the samples produced under conditions of limited potassium supply were lower in weight per 1000 kernels than were the others. The very

low weight of the sample from No. 3 is impossible to explain, unless it was the result of substituting sodium for potassium. As far as the writer knows, there is no reason to suppose that this should make such a great difference, but the work is being extended in the hope of determining more definitely the effect on weight of substituting sodium and calcium for potassium.

The differences in weights of grain as percentages of the total weight are probably not significant except in the case of the complete as compared with the other four samples. The results recorded in Tables IV and VIII show that the extra amounts of nitrogen and ash constituents absorbed by the complete-culture plants and the smaller proportion of these translocated to the kernels as compared with the plants of any of the other cultures, are alone sufficient to account for the differences in the proportion of grain to total weight. There is no evidence that there was greater vegetative growth in the complete than in other cultures.

Total Absorption of Inorganic Constituents

The total amounts of the various inorganic elements absorbed by the plants were calculated from the analyses and the proportions of straw and grain. These calculated values are presented in Table IV as grams absorbed per 100 grams of dry matter. They might have been calculated on the basis of the yields for the various cultures, but these results were considered unreliable and are not recorded.

The complete-culture plants absorbed more of each constituent determined than did any of the other plants, but this result was to be expected on account of the longer absorbing period. The absorption of nitrogen by the plants in the other four cultures was apparently not affected by potassium supply,

TABLE IV

Total absorption of nitrogen and minerals expressed as gm. per 100 gm. dry material

Culture No.	Description of culture	N, gm.	Ash, gm.	K, gm.	Ca, gm.	Mg, gm.	P_2O_5, gm.	SO_4, gm.
1	Complete	1.60*	7.95	2.16	1.40	0.27	0.71	0.54
2	Complete: water	1.07	4.38	1.69	0.73	0.20	0.35	0.16
3	Low K, Na: water	1.10	3.58	0.59	0.92	0.22	0.36	0.24
4	Low K, Ca: water	1.06	3.59	0.58	1.22	0.22	0.54	0.22
5	Complete: K-free: water	1.07	3.31	0.60	0.93	0.21	0.36	0.22

* *This figure excludes nitrate nitrogen absorbed but not reduced. The corresponding figure for total nitrogen is 2.05 gm.*

but the absorption of the ash constituents was. The total amount of ash in complete: water plants was higher than in either of those lots which had a limited potassium supply at all stages, and the ash in these in turn was higher than that in the complete: potassium-free: water plants. Since the potassium uptake of the plants in the last three cultures was the same, it

appears that the time of uptake of this element influences the total absorption of other elements. This conclusion is supported by preliminary results from a study of the distribution of the various elements during the vegetative growth of wheat plants in controlled culture solutions. The increased absorption of other elements brought about by the restriction of potassium supply at any stage of growth is insufficient to compensate for the restricted potassium uptake. Apparently complete absence of potassium from the culture solution from the time the plants are one month old onward paralyzes the absorptive power of the plant to a considerable extent. Whether this is due to the lack of the specific action of the potassium or to an upset balance is not determinable from the results so far obtained.

The absorption of potassium by the plants grown in each of the two low potassium cultures was determined by the potassium supply, and it is quite fortuitous that the plants in the complete: potassium-free: water culture should have absorbed the same amount of potassium in one month as was supplied to the other plants up to the time of heading. Owing to the unreliability of the yields it is impossible to make accurate calculations, but it is evident that practically all the potassium supplied to the two low potassium cultures was absorbed by the plants. The total potassium supplied to each culture during the whole period of development was 0.30 cc. of molar potassium per litre of solution, or 1.35 gm. per tank. The yield of dry matter from each of these tanks was approximately 300 gm. and the total yield of absorbed potassium about 1.75 gm. This is somewhat greater than the amount supplied, but probably the excess can be accounted for by the presence of potassium in the reserves of the seed, and as slight impurities in the other salts.

Considering all but the complete-culture plants, the greatest amount of calcium was absorbed by the plants grown in the low potassium, calcium: water culture, and this was accompanied by the greatest absorption of phosphate. Apparently the sodium supplied to the plants in the low potassium, sodium: water culture did not wholly compensate for the potassium even in absorption, as the calcium uptake of these plants was considerably higher than that of the plants in the complete: water culture. The amount of calcium absorbed by the plants in the complete: potassium-free: water culture was greater than that absorbed by the low potassium, calcium: water plants. Apparently the presence of small amounts of potassium increased the absorption of calcium, but the presence of large amounts depressed it.

There were no significant differences in the amounts of magnesium absorbed by any of the plants grown in tap water after heading. The magnesium level was low in all the samples.

The only significant difference in the phosphate absorptions of the last four samples was in that of the low potassium, calcium: water plants as compared with those of the other three lots. The greater absorption of phosphate has already been shown to accompany the greater absorption of calcium.

The sulphate absorption is apparently increased by limiting the potassium uptake. The amounts absorbed by all lots were small, but the difference between the absorption of the complete: water plants and that of each of the restricted potassium lots is probably significant.

Taken as a whole, the results for total absorption show that the amounts of the inorganic elements absorbed by plants growing in culture solutions are by no means wholly determined by the composition of the solutions. The complete solution in which the complete: water plants were grown until heading contained potassium and calcium in the ratio of 1 : 0.83, and the amounts of these elements absorbed were in the ratio of 1 : 0.43. The low potassium, calcium solution in which the low potassium, calcium: water plants were grown contained potassium and calcium in the ratio of 1 : 50, but the amounts of these elements absorbed were in the ratio of 1 : 2.1. It is obvious that the plants grown in the complete solution exercised a slight, and those in the low potassium, calcium solution a marked, power of selective absorption for potassium.

Composition of Straw and Grain

The results of the analyses of straw and grain are presented in Tables V to VIII. As it is impossible to discuss separately, the results recorded in each table the following paragraphs deal with all results together. In Tables IV, V and VI the figure for nitrogen in complete culture plants excludes nitrates absorbed but not reduced. The mature plants in the other four cultures contained no nitrate nitrogen as they were removed to tap water before reduction of nitrates ceased.

In general, the total absorption by the plant is reflected in the results of the straw composition. Although there were marked variations in the total absorption by the plants in the different cultures, apparently the amount of any one element absorbed did not determine the amount translocated to the kernels except in the case of nitrogen, and to a more limited extent in that of phosphorus. It is evident from the results in Tables V and VI that fairly

TABLE V

Composition of straw and grain expressed as percentages of the dry matter

Culture No.	Description of culture	N	Ash	K	Ca	Mg	P_2O_5	SO_4
Straw								
1	Complete	0.61*	10.70	2.99	2.06	0.33	0.63	0.81
2	Complete: water	0.34	5.67	2.31	1.06	0.23	0.24	0.24
3	Low K, Na: water	0.39	4.40	0.58	1.34	0.26	0.32	0.37
4	Low K, Ca: water	0.36	4.42	0.58	1.86	0.25	0.49	0.36
5	Complete: K-free: water	0.33	4.02	0.62	1.40	0.23	0.23	0.36
Grain								
1	Complete	3.52	2.58	0.54	0.13	0.16	0.84	—
2	Complete: water	2.32	2.14	0.61	0.16	0.17	0.56	—
3	Low K, Na: water	2.32	2.16	0.60	0.20	0.15	0.57	—
4	Low K, Ca: water	2.18	2.28	0.57	0.20	0.17	0.63	—
5	Complete: K-free: water	2.18	2.18	0.57	0.17	0.18	0.57	—

* *This figure excludes nitrates absorbed but not reduced. The corresponding figure for total N is 1.30%.*

constant *amounts* of potassium, calcium and magnesium are tanslocated to the kernels. It is equally evident that the amount of nitrogen in the kernels varies considerably, but from the results in Table VIII it appears that the *proportion* of the organic nitrogen translocated to the kernels is fairly constant. Thus, while the amount of potassium in the kernels from 100 gm. of dry plant material varied only from 0.18 to 0.23 gm., the proportion of the plant's total potassium translocated to the kernels varied from 8.3 to 37.9%; and, while the amount of total nitrogen in the kernels varied from 0.84 to 1.19 gm., the proportion of the plant's total nitrogen in the kernels varied only from 74.6 to 80.4%. The higher percentage of phosphorus in the kernels from complete-culture plants is probably related to the larger amount of protein in these kernels.

TABLE VI

DISTRIBUTION OF NITROGEN AND MINERALS BETWEEN STRAW AND GRAIN FROM 100 GM. OF DRY MATERIAL

Culture No.	Description of culture	Weight of straw or grain, gm.	N, gm.	Ash, gm.	K, gm.	Ca, gm.	Mg, gm.	P_2O_5, gm.	SO_4, gm.
Straw									
1	Complete	66.2	0.41*	7.08	1.98	1.36	0.22	0.42	0.54
2	Complete: water	63.0	0.21	3.59	1.46	0.67	0.14	0.15	0.16
3	Low K, Na: water	63.4	0.25	2.79	0.37	0.85	0.16	0.15	0.24
4	Low K, Ca: water	61.3	0.22	2.71	0.36	1.14	0.15	0.29	0.22
5	Complete: K-free: water	61.1	0.20	2.46	0.38	0.86	0.14	0.14	0.22
Grain									
1	Complete	33.8	1.19	0.87	0.18	0.04	0.05	0.28	—
2	Complete: water	37.0	0.86	0.79	0.23	0.06	0.06	0.21	—
3	Low K, Na: water	36.6	0.85	0.79	0.22	0.07	0.06	0.21	—
4	Low K, Ca: water	38.7	0.84	0.88	0.22	0.08	0.07	0.24	—
5	Complete: K-free: water	38.9	0.85	0.85	0.22	0.07	0.07	0.22	—

* *This figure excludes nitrate nitrogen absorbed but not reduced. The corresponding figure for total nitrogen is 0.86 gm.*

The tendency of all lots of plants to produce kernels with fairly constant mineral contents, accentuates the differences in the proportions of the elements remaining in the straw of the different samples. The complete-culture plants absorbed a little less than four times as much potassium as the plants grown in restricted potassium cultures, but the straw of the former contained more than five times as much potassium as that of the latter.

The results presented in Table VII show that the amounts of the various minerals expressed as percentages of the ash varied markedly for the whole plant and for the straw, but to a much smaller extent for the grain. The variations in the results for the whole plants are of course determined by the factors already discussed in the section on absorption, and those in the results for straw by the relation between total absorption and translocation to the

TABLE VII

MINERALS AS PERCENTAGES OF THE ASH IN WHOLE PLANT, STRAW AND GRAIN

Culture No.	Description of culture	K	Ca	Mg	P_2O_5	SO_4	Recovery of ash
Whole plant							
1	Complete	27.2	17.6	3.4	8.9	6.8	63.8
2	Complete: water	38.6	16.7	4.6	8.1	3.6	71.5
3	Low K, Na: water	16.5	25.7	6.1	10.1	6.6	64.9
4	Low K, Ca: water	16.2	34.0	6.1	15.0	6.2	77.4
5	Complete: K-free: water	18.1	28.1	6.3	10.9	6.8	70.2
Straw							
1	Complete	27.9	19.3	3.0	6.0	7.6	63.7
2	Complete: water	41.0	18.6	4.0	4.0	4.4	71.9
3	Low K, Na: water	13.8	29.1	5.6	7.7	8.8	64.0
4	Low K, Ca: water	13.2	41.5	5.5	11.5	8.3	80.0
5	Complete: K-free: water	15.4	35.2	5.8	5.7	9.0	71.1
Grain							
1	Complete	21.1	4.9	6.4	32.9	—	65.3
2	Complete: water	28.9	7.2	7.8	26.7	—	70.6
3	Low K, Na: water	27.6	9.1	6.7	26.5	—	69.9
4	Low K, Ca: water	25.2	8.7	7.3	27.7	—	68.9
5	Complete: K-free: water	26.4	7.8	8.2	26.1	—	68.5

TABLE VIII

PROPORTION OF ORGANIC NITROGEN AND MINERALS IN GRAIN

Culture No.	Description of culture	Organic N	Ash	K	Ca	Mg	P_2O_5
1	Complete	74.6	10.9	8.3	2.9	18.5	39.6
2	Complete: water	80.4	18.0	13.6	8.2	30.0	58.3
3	Low K, Na: water	77.3	22.1	37.3	7.6	27.3	59.2
4	Low K, Ca: water	79.2	24.5	37.9	6.6	31.8	45.2
5	Complete: K-free: water	79.4	25.7	36.7	7.5	33.3	61.2

kernels. The higher percentage of phosphate in the ash from complete-culture kernels, which has already been attributed to the higher protein level of these kernels as compared with that of the others, reduces the percentages of all other elements as calculated on the basis of the total ash.

NITROGEN DISTRIBUTION AND QUALITY OF GRAIN

The results presented in Tables IX to XI were obtained in studies carried out in an effort to determine the quality of the grain. The individual samples were too small to be studied by the methods usually employed in determining quality, so a detailed study of the nitrogen distribution in the grain, and of the chemical and physical nature of the gluten, was undertaken.

The results of the studies on the distribution of gluten and non-gluten nitrogen in the kernels are recorded in Table IX. These results confirm the

conclusion reached in an earlier paper (11) that the distribution of the nitrogen fractions is directly related to the total nitrogen content of the kernels. It is obvious that the gluten nitrogen is the fraction which increases with an increase in total nitrogen, as there is a spread of only 0.09% in the non-gluten nitrogen values expressed as percentages of the dry matter, as compared with a spread of 1.31% in the gluten nitrogen values on the same basis.

Although there are only two samples, there is an indication that restricting the potassium supply may result in a slight increase in the non-gluten nitrogen.

TABLE IX

DISTRIBUTION OF GLUTEN AND NON-GLUTEN NITROGEN IN GRAIN

Culture No.	Description of culture	Total N % D.M.	Gluten N		Non-gluten protein N		Non-protein N		*Total* non-gluten N	
			% T.N.	% D.M.	% T.N.	% D.M.	% T.N.	% D.M.	% T.N.	% D.M.
1	Complete	3.52	77.1	2.71	16.3	0.57	6.6	0.23	22.9	0.80
2	Complete: water	2.32	68.8	1.60	24.2	0.56	7.0	0.16	31.2	0.72
3	Low K, Na: water	2.32	65.0	1.51	27.4	0.63	7.6	0.18	35.0	0.81
4	Low K, Ca: water	2.18	64.3	1.40	28.4	0.62	7.3	0.16	35.7	0.78
5	Complete: K-free: water	2.18	64.2	1.40	28.8	0.63	7.0	0.15	35.8	0.78

The kernels from the low potassium, sodium: water plants contained a higher proportion of the total nitrogen and of the dry matter in the form of non-gluten nitrogen than did the kernels from the complete: water plants, although the total nitrogen contents were the same.

The results presented in Table X give some indication of the quality of the grain produced by each of the cultures. The gluten weights are largely determined by the gluten nitrogen content, but the wet glutens from samples which are lower in gluten nitrogen are slightly more than correspondingly low in weight. The dry matter contents of the various glutens show no significant variations, nor do the total nitrogen contents except in the case of the gluten from the complete: potassium-free: water as compared with the other samples. The higher percentage of nitrogen in this sample can be attributed to the coarseness of the wet gluten. It has been noted in an unpublished study that the texture of the gluten determines, to some extent at least, the ease with which the starch is washed from the dough ball in the gluten washing process. Within a particular series, the coarser gluten has the higher percentage of nitrogen due to the more complete removal of starch in a definite time.

The estimation of the quality of the various samples was necessarily based to a large extent on the characteristics of the wet gluten samples. These samples were obtained by the procedure recommended by Dill and Alsberg

(3) except that wheat meal, ground to pass a 1 mm. sieve, was used instead of flour. Admittedly this estimate is subject to all the errors of personal opinion, but the variations among the physical characters of the various glutens were so marked that there is little doubt as to the variations in quality. The dough ball made from grain grown in complete culture handled easily at all stages of the washing process, and yielded a gluten of excellent texture, elasticity and color. The dough ball made from grain grown in complete: potassium-free: water culture was very slack and hard to handle in washing, and yielded a gluten which was coarse, non-elastic and dark. The other samples were intermediate between these two in all respects, the differences being recorded in Table X.

TABLE X

WEIGHT AND NITROGEN CONTENT OF GLUTEN

Culture No.	Description of culture	Weight of gluten from 10 gm.			Nitrogen in gluten		Nature of wet gluten
		Wet, gm.	Dry, gm.	Dry as % of wet %	Wet, %	Dry, %	
1	Complete	7.10	2.39	33.7	3.82	11.2	Excellent in all respects.
2	Complete: water	4.05	1.38	34.1	3.94	11.6	Good in all respects.
3	Low K, Na: water	3.91	1.35	34.5	3.85	11.2	Fairly good; somewhat coarse.
4	Low K, Ca: water	3.57	—	—	3.93	—	Fairly good; somewhat coarse.
5	Complete: K-free: water	3.31	1.12	33.8	4.23	12.4	Poor; coarse, spongy and dark.

It is of interest to note that the complete: water culture plants did not yield as good a gluten as the complete culture plants. In the earlier paper (11) it was concluded that nitrogen supply alone did not affect the quality of the gluten. In that study all the elements of the complete culture solution except nitrogen were supplied to the limited nitrogen plants after heading, but in the present study the corresponding sample was grown in tap water. Apparently even during the later stages of development the continued

TABLE XI

NITROGEN FRACTIONS OF GLUTEN EXPRESSED AS PERCENTAGES OF GLUTEN NITROGEN

Culture No.	Description of culture	Nitrogen fractions of gluten						
		Humin	Amide	Arginine	Basic	Filtrate from basic	Mono-amino	Recovery
1	Complete	1.7	22.0	10.4	16.5	61.0	49.9	101.2
2	Complete: water	1.9	21.5	9.2	14.9	63.3	48.9	101.6
3	Low K, Na: water	2.0	21.8	8.6	15.0	62.7	49.4	101.5
4	Low K, Ca: water	1.6	21.6	—	—	—	—	—
5	Complete: K-free: water	1.5	21.8	8.2	14.7	63.0	49.5	101.0

absorption of mineral elements is beneficial to the quality of the grain produced, even though no nitrogen is absorbed during these stages.

The results of the fractional analyses of gluten nitrogen (Table XI) indicate that the physical nature of the gluten is probably not determined by the chemical composition. This is in agreement with the results of many other workers. There is a possibility that the variations in the basic nitrogen fraction, and particularly arginine, may be significant, but this is impossible to determine from the results of the present limited study.

Discussion

A review of all results presented shows that the amount and nature of the minerals absorbed and the time of uptake influence the character of the grain produced by the plant. There is no doubt that the complete culture sample is the best in quality, and the differences between it and the complete: water culture sample demonstrate the value of minerals absorbed after the time of heading.

In evaluating the effect of restricted potassium supply, the complete: water sample must be considered as the control. A comparison of the low potassium, sodium: water sample and the control indicates that potassium cannot be wholly replaced by sodium either in absorption or in functions within the plant. The use of sodium is apparently somewhat beneficial as compared with complete replacement of potassium by calcium.

The poorer quality of the grain from low potassium, calcium: water and complete: potassium-free: water cultures might be the result of limited potassium supply or of too great an absorption of calcium. It is believed that a comparison of the absorption of these two samples shows that the former is the more important cause of the poor quality. The low potassium, calcium: water plants absorbed 1.22 gm. of calcium per 100 gm. of dry matter, and the complete: potassium-free: water plants absorbed 0.93 gm. Thus, although the former produced grain of decidedly better quality than the latter, the calcium uptake was about 30% greater, while the amounts of potassium and nitrogen absorbed were the same. Had the extra calcium been the cause of the poorer quality, then the quality of the grain from the complete: potassium-free: water plants would have been better than that of the grain from the low potassium, calcium: water plants.

These two samples also serve to illustrate the effect of the time of absorption of potassium. Although the total absorption was the same, the low potassium, calcium: water plants received potassium in small amounts supplied regularly from germination until the time of heading, and the other plants received it in the complete culture for one month. The difference in quality of grain from the two lots must have been the result of the time of absorption of potassium as the other factors determining development were the same.

The results of the analyses of the grain serve to emphasize the fact that it is impossible even to estimate the amounts of the various nutrients absorbed by studying the distribution of these nutrients in the grain alone. Total

nitrogen determinations of the grain give a good estimate of the nitrogen absorbed by the plant, because a fairly constant proportion of the plant's total nitrogen is translocated to the kernels. The minerals, on the other hand, are translocated to the kernels in fairly constant amounts per unit of dry weight. It therefore appears necessary, in any nutritional study, to determine the distribution of the various elements in the vegetative parts of the plant as well as in the kernels.

This investigation in somewhat modified form is being repeated and the absorption and distribution of the various elements at several stages of development are being determined. More definite measures of quality are planned, and it is hoped that the quality of the samples may be definitely and quantitatively related to the cultural treatment.

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NATURAL AND ARTIFICIAL HYBRIDIZATION OF *AVENA SATIVA* WITH *A. FATUA* AND ITS RELATION TO THE ORIGIN OF FATUOIDS[1]

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Abstract

The common fatuoid or false wild oat is an aberrant grain type which occurs frequently in cultivated oats. Three theories have been advanced in explanation of the origin of fatuoids, namely, chromosome aberration, gene mutation, and natural hybridization. The third theory has been much criticized in recent years. Observations in Alberta, Canada, indicated the possibility of natural hybridization between *Avena fatua* and *A. sativa* being an important factor in the origin of fatuoids. Genetic studies were made of both artificial and natural crosses between *A. fatua* and *A. sativa* which indicate the probability that the common fatuoid is a normal Mendelian segregate from the crosses in question. It is believed that more or less complete selective elimination of non-fatuoid segregates can be explained. While the authors believe that natural hybridization is the usual means by which fatuoids originate, they, nevertheless, entertain the possibilities of origin by chromosome aberration or by gene mutation.

Introduction

The origin of fatuoids, or false wild oats, has been the subject of controversy for nearly fifty years, and remains still an unsettled problem. Involved in the solution of this problem are implications both of scientific and economic importance. Accurate knowledge of the mode of origin of fatuoids might reasonably be expected to elucidate, to some extent, the general problem of variation; and also to point the way to methods for the eradication or control of fatuoids in seed stocks.

Huskins (14, 15) has classified several types of fatuoids on the basis of chromosome numbers and meiotic behavior. The present paper is concerned only with Huskins' so-called type 1, or "A" series which presumably carries the normal chromosome complement, and which will be referred to herein under the term, "fatuoid". This fatuoid may be described as follows:

The common fatuoid is an aberrant grain type occurring in cultivated oats. The homozygous form differs from the cultivated plant in three main characters of the grain, namely, in articulation, pubescence and awn development. A large horseshoe-shaped "sucker mouth" articulation is present on all grains of the spikelet; dense bushy pubescence is present on both callus and rachilla; a strong, twisted and geniculate awn is present on each grain of the spikelet. The heterozygous form is, with respect to these characters which comprise the "fatuoid complex", intermediate between the homozygous

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form and the cultivated variety in question. It segregates in the ratio of approximately 1 homozygous fatuoid : 2 heterozygous fatuoids : 1 normal cultivated type; all segregates being, apparently, of equal vigor.

This is the fatuoid type commonly found to be of practical significance, other types being of very rare occurrence. While the occasional occurrence of fatuoids is not of great economic importance in ordinary commercial oat fields, it is, nevertheless, very troublesome in plots and fields of "Registered" and other high grades of seed oats.

In the course of investigations relating to the production of "Elite" and "Registered" seed stocks of oats at the University of Alberta in 1923-24, Huskins and Fryer (12) and Huskins (13) studied the genetic and cytological aspects of the origin of fatuoids, and came to the conclusion that "the evidence is overwhelmingly against the theory that fatuoids arise by natural crossing", and, "that some chromosome aberration, rather than a change in a single gene, is instrumental in causing their appearance." Later conclusions by Huskins, based on further genetic and cytologic investigations (14, 15, 16), are essentially the same as the above statement.

In recent years it has become increasingly evident from work at the University of Alberta with the same seed stocks as those used by Huskins and Fryer (12, 13), and from studies of commercial stocks produced by several registered seed growers, that fatuoids may originate also by means other than gene mutation or chromosomal aberration. In the seed stocks from which fatuoids have been collected, there occurred other off-types which appeared to be segregates such as one might expect from crosses between *Avena fatua* and *A. sativa*. During the winter of 1928-29 our attention was drawn by Mr. N. Lewis of the Dominion Seed Branch to a similar series of off-types occurring together with fatuoids and true wilds in registered seed stocks in the Provincial Seed-cleaning Plant at Edmonton. During the following summer (1929) numerous fields of oats were examined, and in a number of cases similar series of off-types were found, almost invariably in association with fatuoids and true wilds.

Although some of the more recent writers (14) have stated that the natural crossing theory of origin of fatuoids is untenable, the circumstantial evidence and the material at hand pointed so strongly to such an origin that further studies on the relation of crosses between *A. fatua* and *A. sativa* to the origin of fatuoids seemed to be warranted.

The studies reported herein consist of genetic analyses of the off-types found occurring naturally in registered seed stocks and fields of oats, and of an artificial cross between *A. fatua* and *A. sativa*. Since the off-types found occurring naturally appeared to be segregates from natural crosses between *A. fatua* and *A. sativa*, they have been compared with artificial hybrids from these two species with reference to morphological characteristics and segregation ratios.

Review of Literature on the Origin of Fatuoids

The earliest report of a direct study on the origin of fatuoids is that of Haussknecht, published in 1884 and cited by Zade (30). Haussknecht observed, in Thuringia, a continuous series of forms between *Avena sativa* and *A. fatua*, which he called *A. fatua* var. *transiens* or "Zwischenformen". He believed, in accord with the general belief of that time, that these forms could not have arisen from interspecific hybridization as no diminution of fertility was observed. Evidently Haussknecht believed, as did many botanists of his day, that sterility was the criterion of hybridity. The objection to the acceptance of intermediate forms because of the lack of sterility would not be valid at the present time, since it is generally accepted that hybrids between species are not necessarily sterile.

Koernicke and Werner, in a paper published in 1885, reviewed by Stanton, Coffman and Wiebe (26), reported studies on intermediate forms similar to those studied by Haussknecht which, they concluded, originated through natural interspecific hybridization. This direct contradiction of Haussknecht's conclusion marks the beginning of the controversy regarding the origin of fatuoids.

In 1900 Fischer (26) reported extensive investigations on intermediate forms. He found that these types did not possess the property of delayed germination as did *A. fatua*. He observed that intermediate forms appear "spontaneously and quickly among the weakly haired and awned, gray-kernelled winter oat", and seriously questioned their origin through natural crossing.

In the reports of Nilsson-Ehle published in 1907 and in 1911 (26) these intermediate forms were referred to for the first time as "fatuoids". The theory was advanced that the fatuoid probably originated through a loss-mutation in cultivated oats.

During the period from 1907 to 1912 Criddle (3, 26) published four papers on "white wild oats" or "false wild oats". He gave valuable information on the characteristics of fatuoids and concluded that they originated by mutation.

Zade, according to Stanton, Coffman and Wiebe (26), reported in 1912 the results of experiments with "Zwischenformen" and stated that these types originated by natural crossing between *A. fatua* and *A. sativa*. In 1914 Tschermak (26) came to the same conclusion after similar experiments.

This brief review of the earlier work is probably sufficient to recall the historical development of some of the present viewpoints. For more complete reviews, the reader is referred to Stanton, Coffman and Wiebe (26), and to Huskins and Fryer (12). In order to present more comprehensively the different hypotheses which have been advanced with reference to the origin of fatuoids, the more recent contributions will be reviewed in relation to the particular hypothesis to which each author subscribes.

Natural Crossing Hypothesis

In his book "Der Hafer", published in 1918, Zade (30) discussed his investigations with fatuoids. He stated that grain types intermediate between *A. fatua* and *A. sativa*, and of brown or yellow color, are frequently found in samples of threshed oats. In the progeny of these intermediates he observed segregation of types in the ratio, 1 *sativa* : 2 intermediate : 1 *fatua*. His description of these indicates that his *sativa*, intermediate and *fatua* types are probably the same as the types that more recent writers would call normal cultivated, heterozygous fatuoid, and homozygous fatuoid types, respectively. Zade believed that these types arose as segregates from natural crosses between *A. fatua* and *A. sativa*. The segregation from this cross, however, was not a monohybrid segregation such as was observed when the intermediates were grown. To explain this difference in breeding behavior Zade assumed that the intermediate forms were segregates, heterozygous for one factor, from a cross which had occurred several years previously. Thus, the generations from the intermediates were not F_2, F_3, etc., but F_{x2}, F_{x3}, etc. In some cases intermediate forms produced polyhybrid segregation in the F_{x2}, but, unfortunately, most of these plants were destroyed by insects.

Zade believed that further evidence in favor of the natural crossing hypothesis is provided by the fact that the intermediate types were of frequent occurrence, while mutations are known to occur very rarely. Examination of large samples of threshed seed always showed intermediate forms associated with wild oats. A positive correlation was found to exist between the number of intermediate type grains and the number of wild type grains in a given sample.

Challenged by the conclusions of Nilsson-Ehle (26), Zade refused to accept the former's statement that wild oats do not occur in the vicinity of Svalöf, Sweden (a fact submitted by Nilsson-Ehle as conclusive proof against the natural crossing hypothesis). He also pointed out that Nilsson-Ehle's seed stocks were not necessarily pure lines.

Crépin (1) studied the breeding behavior of an intermediate type plant found in a field of cultivated oats, and concluded that it was a natural hybrid of *A. fatua* and *A. sativa*. He believed that natural crossing, though of comparatively rare occurrence, accounted for the presence of intermediate types which cause farmers to say that cultivated oats degenerate into wild oats. In a later paper (2) he reported that he obtained an intermediate form similar to that described above, as the F_1 of a cross between *A. fatua* and *A. sativa*. In the F_2, segregation occurred in the ratio of 1 *fatua* : 2 intermediate : 1 *sativa*. This behavior corresponds to that observed in the generation arising from the heterozygous fatuoid.

In 1929 Tschermak (28)* published data from the study of several crosses between *A. sativa* and *A. fatua* which he believed strongly favored the natural crossing hypothesis. The *sativa* varieties that he used were Maunthers,

* *We are indebted to Dr. V. H. Florell for an English translation of this paper.*

Svalöf's Victory, Daubeney and Ligowa. The last-named variety is a side oat. He did not describe the wild parent; but the text suggests that two types were used, a non-yellow (black or brown) type with pubescent lemmas, and a yellow type with glabrous lemmas. The latter type must have closely resembled a homozygous fatuoid. The F_1 plants, being similar in all crosses, are described collectively. With respect to grain characters, the F_1 individual was more or less intermediate between the parental forms. The articulation was similar to that of the cultivated parent; awn development was fairly strong and confined almost entirely to the lower floret; pubescence of the lemma was always present to an intermediate degree; pubescence of the callus was in the form of two tufts of long and short hairs; the rachilla was, as a rule, glabrous. Summarizing, Tschermak stated that in all descriptions, except in the case of lemma pubescence, the F_1 type of grain corresponded to the accepted description of the heterozygous fatuoid. Color of lemma in the F_1 was not described; but we must infer, from the comparison with the heterozygous fatuoid, that it corresponded to that of the cultivated parent.

In the F_2 of each cross Tschermak found but three types of segregates, namely, *fatua*, intermediate and *sativa* forms, occurring in the ratio of 4 : 9 : 3. (He had evidently observed a similar segregation ratio in the generation arising from the heterozygous fatuoid.) The F_2 data of the four crosses are summarized as follows:

	fatua	intermediate	*sativa*
Combined F_2 data	158	444	132
Calculated ratio	4	9	3
Observed ratio	3.20	9	2.67

Tschermak stated that, while the F_3 population was too small to give ratios positively demonstrating a two-factor basis of inheritance, it served, nevertheless, to validate certain conclusions. Awnless, glabrous *sativa* forms bred true; *sativa* forms with a degree of awning and pubescence segregated in the ratio, 3 *sativa* : 1 *fatua*. The *fatua* forms were constant. The F_2 intermediate phenotype was shown to be expressed by four different genotypes: four-sixteenths of the F_2 intermediates produced *fatua*, intermediate and *sativa* forms in the ratio of 4 : 9 : 3; two-sixteenths produced intermediate and *sativa* forms in the ratio of 3 : 1; one-sixteenth remained constant.

A trifactorial inheritance scheme was devised. The genotype *aabbCC* was ascribed to the *sativa*, and *AABBCC* to the *fatua* parent. The following factorial interactions were assumed: *AA* gives *fatua* phenotype; *Aa* is hypostatic to *B*(*BB* or *Bb*); *B*(*BB* or *Bb*) with *Aa*, or alone, gives the intermediate phenotype; *C* is hypostatic to *AA*, *BB*, *Bb* and epistatic to *Aa* and gives the *sativa* phenotype.

Mutation Hypothesis

As stated by Nilsson-Ehle (21) in 1921, the mutation hypothesis assumes that there occurs in one of the gametes a mutation affecting the gene complex which governs the characters of the fatuoid complex. Such an abnormal

gamete, mating with a normal one, gives rise to a heterozygous fatuoid plant. This plant when selfed produces normal, heterozygous fatuoid, and homozygous fatuoid plants, in the ratio of 1 : 2 : 1.

Jones (19) has recently (1930) advanced a modified mutation hypothesis based on Huskins' (15) $\frac{ABC}{ABC}$ formula for normal *A. sativa.* (See chromosome aberration hypothesis below.) This hypothesis assumes that chromosome B carries the factors for *fatua* grain characters, and that chromosome C carries factors for *sativa* grain characters; the factors on C being epistatic to those on B. Mutation in C removes this epistasis, and thereby allows factors in B to express *fatua*-like grain characters.

Nishiyama (22) concluded in 1931, after extensive genetic and cytological observations, that the common fatuoid, with the normal chromosome number, originates by mutation in the C chromosome; and that fatuoids which show deviations from the normal chromosome number [*i.e.*, Huskins' (15) B and C series, Goulden's (10) families II and III, and Nishiyama's (22) strains H_{20}-35 and H_{58}-12] originate through chromosome aberrations. More recent studies by Nishiyama (23) have confirmed his earlier viewpoint and that of Jones (19). His recent report claims to have established a synoptic gene, or genes, which have their loci in the longer arm of the C chromosome (F_1). This "discovery of a chromosome or gene controlling the meiotic behavior has great significance in the cytology of hybrids, where phylogenetic relation of parents is often represented by the degree of synoptic affinity."

Chromosome Aberration Hypothesis

The chromosome aberration hypothesis, stated by Huskins (13, 14, 15) as an explanation of the origin of fatuoids, is based upon Winge's (29) explanation of the origin of speltoids in wheat, and like the latter involves a consideration of the phylogeny of the species concerned. The higher species of *Avena* and *Triticum* are believed to have evolved by multiplication of the chromosomes of more primitive species. In *Avena* the hexaploid species *A. sativa*, which contains most of our common cultivated varieties, has 21 pairs of chromosomes representing three sets of each of the seven chromosome pairs of the diploid species. The set, or di-triploid group, of chromosomes concerned with grain characters is represented in *A. sativa* by the formula $\frac{ABC}{ABC}$. The $\frac{B}{B}$ pair differs from the $\frac{C}{C}$ pair only in the factor or factors affecting articulation, pubescence, and awn development of the grain. $\frac{B}{B}$ carries factors which determine fatuoid grain characters; $\frac{C}{C}$ carries factors which determine normal grain characters; and $\frac{C}{C}$ is epistatic to $\frac{B}{B}$. Normal pairing of A with A, B with B, and C with C will maintain the equilibrium of the groups,

and true-breeding progeny will be produced. But, if faulty pairing takes place occasionally between B and C, gametes of the types ABB and ACC will result. The mating of ABB with the normal ABC gamete gives an $\frac{ABC}{ABB}$ zygote. It is assumed that this zygote, having an extra "dose" of fatuoid factors, develops into a heterozygous fatuoid. The resulting individual, when selfed, will segregate for normal $\frac{ABC}{ABC}$, heterozygous fatuoid $\frac{ABC}{ABB}$ and homozygous fatuoid $\frac{ABB}{ABB}$ types in the ratio of 1 : 2 : 1, respectively.

Huskins (15) describes three types of fatuoids: in A series the heterozygous type has 42 chromosomes and segregates in the ratio of 1 : 2 : 1 for normals, heterozygous fatuoids and homozygous fatuoids, all segregates being of normal 42-chromosome complement; in B series the heterozygote segregates for normals, heterozygotes and fatuoids having 42, 41 and 40 chromosomes, respectively; in C series the heterozygote segregates for normals, heterozygotes and fatuoids having 42, 43 and 44 chromosomes, respectively.

For more complete reviews of the recent literature bearing directly on the various hypotheses, the reader is referred to Jones (19) and Nishiyama (22).

Materials

Two distinct lots of material were studied. One lot consisted of F_1, F_2 and F_3 plants from an artificial cross between *A. fatua* and *A. sativa*. The other lot consisted of aberrant grain types found in threshed seed stocks and off-type plants selected in the field. The progenies of the latter group were studied in the same manner as those of the artificial crosses.

The artificial crosses were made in 1929. Reciprocal crossing was performed, but success was attained only where *A. fatua* was the pistillate parent. The failure to obtain seed in the reciprocal cross does not bear any special significance, since only a small number of cross fertilizations were attempted. Johnson (18) when continuing these studies found no difficulty in obtaining reciprocal crosses. Surface (27) found that there were no differences in either the F_1 or F_2 from reciprocal crosses between wild and Kherson oats.

The *A. fatua* parents used in these crosses were stray plants of the common wild oat, which grew adjacent to cultivated oat varieties on the plots at the University of Alberta. Seeds of each of these plants were collected and grown separately in parental check plots for three years. The type appeared to be uniform and free from genetic variation. The grain of *A. fatua* is characterized by an oblique, "suckermouth" articulation, heavy pubescence on rachilla, callus and lemma, dark brown or black lemma color, and strong geniculate awns on all grains of the spikelet. (Plate 1, *A*.) Other characteristics which distinguish this wild form are its delayed germination, when matured under certain environmental conditions, its early maturity, the ease with which it shatters at maturity and the fine drooping branches of its panicle.

The *A. sativa* parent, Selection No. 76, was sent to the University of Alberta from England in 1927 by Dr. C. L. Huskins, who had previously obtained it from Professor Shegalov of Russia. This variety came to us with the distinction of having never produced awned grains nor given rise to fatuoids. It has been tested over a period of five years at the University of Alberta in one-tenth acre plots, and in several hundreds of check rows; and still retains that distinction. It has also been tested by T. R. Stanton* at Aberdeen, Idaho, for a more limited period, with similar results. Selection No. 76 is an early maturing oat of good agronomic quality and of fair yielding capacity. The plant is short, with a relatively small, medium spreading panicle; the lemma is white and glabrous; the rachilla ranges from glabrousness to a very sparse, short pubescence; the callus exhibits sparse, short to medium pubescence; the articulation is small and not oblique (Plate 1, *B*).

The natural aberrant material was obtained from elite stock of Victory maintained at the University of Alberta, and from elite stock of Banner maintained by Mr. Nels Linden at Wetaskiwin, Alberta. Two generations of the former and one of the latter material have been grown and analyzed. Other reference to this material has been made in the introduction.

Methods

The crosses were made in the summer of 1929 and four F_1 plants were grown in the greenhouse during the winter of 1929-30.

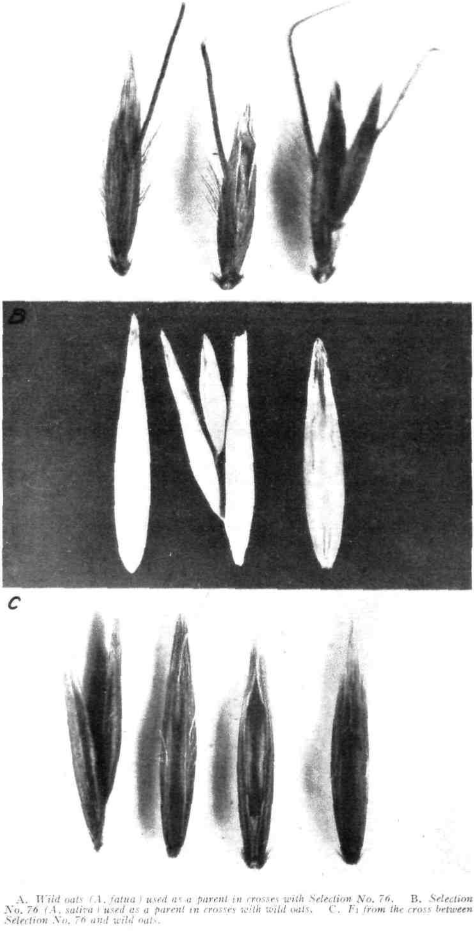
The F_2 consisting of 69 plants was grown as a separate population. Parental check plots were grown adjacent to the hybrid plots in order to provide comparable material for study.

Each individual F_2 plant was thoroughly examined and classified for all the major segregating characters, especially those concerned with the fatuoid complex.

The four F_2 populations showed the same general type of segregation as far as could be expected with the limited numbers, and consequently were treated as one group in the final analysis. In all there were 69 F_2 plants. A detailed description of each plant is presented in Table X.

The F_3 was grown in the greenhouse during the summer of 1931. Thirty seeds from each F_2 plant were sown in sterilized soil in wooden flats, together with seed of both the cultivated and wild parents. The objective of this plan, in addition to providing comparative material in the morphological studies, was to determine, if possible, whether delayed germination was associated with any of the segregating characters, especially those of the fatuoid complex. Seedling emergence notes were made on each hybrid and parental plot. The number of plants in some of the F_3 lines was too small to be significant, but they were sufficiently indicative to aid in determining the genotype of the individual F_2 plants and the validity of the hypothesis used to explain the genetic ratios obtained in the study reported herein.

**From correspondence with Mr. T. R. Stanton.*



A. *Wild oats (A. fatua) used as a parent in crosses with Selection No. 76.* B. *Selection No. 76 (A. sativa) used as a parent in crosses with wild oats.* C. *F_1 from the cross between Selection No. 76 and wild oats.*

In all generations each plant was harvested separately, and retained intact in an individual envelope. When the seeds were removed from F_2 plants for planting, a reserve supply of seed from each plant was retained. These reserves have, by providing a means for the re-examination of the progenitors, proved to be very valuable in the analysis of F_3 families. Many difficulties encountered in the original analysis of the F_2 were cleared up when these reserves were re-examined in the light of their breeding behavior in the F_3. The same general method was followed in handling the natural aberrant material.

Experimental Results

The Artificial Cross *A. fatua* × *A. sativa*

The four F_1 plants showed a marked uniformity for all readily observable characters, the expressions of which were more or less intermediate between those of the parents. Conditions for growth were not very favorable in the greenhouse, consequently only a small number of seeds was produced by the F_1 plants. No sterile spikelets were noted. The black lemma color of the wild parent tended to be dominant, the F_1 expression being brown to dark brown. Awn development ranged from weak to strong on the primary grain, but in no case extended to the secondary grain. Rachilla pubescence varied from complete absence to an intermediate condition. Callus pubescence was expressed as sparse to medium dense tufts of short to medium-long hairs on the primary grain, but was not expressed on the secondary grain. Lemma pubescence was present on the primary grain as sparsely scattered hairs of short to medium length, but did not occur on the secondary grain. The articulation, while tending to resemble the cultivated rather than the wild type, was intermediate in many respects; it did not possess the "sucker mouth" nor the easy-shattering character of the wild oat, yet it had a far larger abscission surface and a looser connection than the cultivated oat; the plane of its position was not at a right angle to the long axis of the grain as in *A. sativa*, but tended to be oblique as in *A. fatua* (Plate 1, *C*).

In the first segregating generation (F_2) types were produced exhibiting practically all combinations between the parents in lemma color, awn development and pubescence. In the case of articulation, however, there were produced three more or less definite types, *fatua*, intermediate and *sativa*. The *fatua* or "suckermouth" type was always unmistakable. The difference between intermediate and *sativa* types was mainly a matter of size, consequently distinction between them was not always clear. F_3 breeding behavior, however, served definitely to establish these types. Since the primary objective of this investigation was to study the mode of inheritance of the fatuoid complex, only a brief statement will be made of the mode of inheritance of other characters that segregated and were studied. It can be stated that the genetic ratios obtained for each character when studied independently conforms, in general, with the results reported by other investigators; consequently it seems unnecessary to burden the reader with a detailed literature review.

Lemma Color

The grain of the F_1 plants was reddish brown to reddish black. There was considerable variation, but in no case did the color appear to be as dark as that of the wild parent.

The grain of the F_2 plants was placed in three classes, namely, black, gray and white. Di-hybrid ratios were obtained, and the numbers of plants of the respective colors agreed closely with expectation. The data obtained are compared with the theoretical expected in Table I.

TABLE I

SEGREGATION FOR COLOR IN F_2 OF A CROSS BETWEEN *A. sativa* AND *A. fatua*, AND COMPARISON WITH A THEORETICAL 12 : 3 : 1 RATIO BY THE χ^2 METHOD

Class	F_2 frequencies		O−C	$(O-C)^2$	$\frac{(O-C)^2}{C}$
	Observed	Calculated			
Black	54	51.7	2.3	5.29	.102
Gray	11	13.0	2.0	4.00	.308
White	4	4.3	0.3	.09	.021
Total	69	69.0			χ^2 = .431

P = < 0.61

That the actual ratio obtained is a good fit to the theoretical expected is indicated by the P value of .61. The *fatua* parent evidently contained a pair of dominant factors for black (*BB*), and a pair of factors for gray (*GG*) which are hypostatic to the factors for black. The white (*bbgg*) segregate in the F_2 is recessive to both black and gray.

The breeding behavior of the F_2 plants in the F_3 was in accordance with the theoretical expectation, except for the occurrence of too many white segregates and too few of the gray segregates. Between these two colors there were several shades showing all gradations and making definite classification difficult. Other workers have also experienced difficulty in distinguishing between these two colors in classifying oat grains. Some plants which showed no trace of the gray color on the dorsal side, show a distinct color on the ventral (palea) side. Such plants also showed a darker gray on the upper grains of the spikelet than on the lower grains. These plants were usually found to be heterozygous. The homozygous grays had a more distinct color. One of the gray *fatua* segregates, which appeared homozygous for gray in the F_3 under field conditions, produced both white and gray types when grown in the greenhouse. In view of these facts it appeared safe to conclude that it was impracticable to distinguish between white and gray grain color. This was especially true in the case of the heterozygous gray types and in such cases it was necessary to group the data from gray and white grains.

Rachilla Pubescence

In the F_1 the rachilla of the lower grain was practically smooth with only an occasional short hair on some of the grains. Rachilla pubescence of the F_2 plants was divided into three classes: long and dense, as in the *fatua* parent; short and sparse; and absent, as in the *sativa* parent. Since the heterozygote varied from smooth to a condition of only a few short sparse hairs, as was indicated in the F_1, it was difficult to separate the intermediate from the *sativa* type. When these two groups were combined, a simple monohybrid ratio was obtained. The data obtained are compared with the theoretical expected in Table II.

TABLE II

SEGREGATION FOR RACHILLA PUBESCENCE IN F_2 OF A CROSS BETWEEN *A. sativa* AND *A. fatua*, AND COMPARISON WITH A THEORETICAL 3 : 1 RATIO

Class	F_2 frequencies		Dev.	P.E.	D/P.E.	Odds
	Observed	Calculated				
Smooth	53	51.75	1.25	2.43	0.514	<1 : 1
Hairy	16	17.25				
Total	69	69.00				

That the actual ratio obtained is a good fit to the theoretical expected, is indicated by odds of less than 1 : 1. The simple ratio indicates the presence of a single factor pair governing rachilla pubescence, with smooth rachilla dominant.

Callus Pubescence

In the F_1 the callus had medium-dense tufts of short to medium-long hairs on the primary grain, and none on the secondary grain. In the F_2 the plants were placed in three classes of callus pubescence, namely: long, dense, tufted pubescence as in the *fatua* parent; intermediate pubescence as in the F_1; and absence of pubescence as in the *sativa* parent. The segregation in the F_2 is that of a simple monohybrid. The data obtained are compared with the theoretical expected in Table III.

TABLE III

SEGREGATION FOR CALLUS PUBESCENCE IN F_2 OF A CROSS BETWEEN *A. sativa* AND *A. fatua*, AND COMPARISON WITH A THEORETICAL 1 : 2 : 1 RATIO BY THE χ^2 METHOD

Class	F_2 frequencies		O−C	$(O-C)^2$	$\frac{(O-C)^2}{C}$
	Observed	Calculated			
Hairy	20	17.25	2.75	7.56	.438
Intermediate	32	34.50	2.50	6.25	.180
Smooth	17	17.25	.25	.06	.004
Total	69	69.00			χ^2 = 0.622

P = <0.61

That the actual ratio obtained is a good fit to the theoretical expected ratio is indicated by the high P value of .61. The *fatua* parent contains a factor for callus pubescence which is intermediate in the heterozygote and practically dominant to the factor for absence of callus hairs in the *sativa* parent.

Lemma Pubescence

In the F_1, lemma pubescence was expressed as an intermediate condition between that of the two parents. Sparsely scattered hairs of short to medium length were present on the primary grain, but absent from the secondary grain. In the F_2, the plants were placed originally in classes similar to those of the parents and in two intermediate classes, one with sparse medium-length hairs and the other with sparse short hairs. There was, however, some difficulty in differentiating between the intermediate types and the fully pubescent types in some cases, so they were all grouped in the pubescent class. The segregation in the F_2 is that of a simple monohybrid. The data obtained are compared with the theoretical expected in Table IV.

TABLE IV

SEGREGATION FOR LEMMA PUBESCENCE IN THE F_2 OF A CROSS BETWEEN *A. sativa* AND *A. fatua*, AND COMPARISON WITH A THEORETICAL 3 : 1 RATIO

Class	F_2 frequencies		Dev.	P.E.	D/P.E.	Odds
	Observed	Calculated				
Hairy	50	51.75	1.75	2.43	0.720	<1 : 1
Smooth	19	17.25				
Total	69	69.00				

That the actual ratio obtained is a good fit to the theoretical expected is indicated by the odds of less than 1 : 1. The *fatua* parent contains a factor for lemma pubescence, which is intermediate in the heterozygote and partially dominant to the factor for absence of lemma pubescence in the *sativa* parent.

Awn Development

The primary grains of the F_1 plants had weak to strong awns, but the secondary grains were completely awnless. In the F_2 the plants were classified according to whether the kernels were free of awns like the *sativa* parent, intermediate as in F_1, or strong and geniculate as in the *fatua* parent. The segregation in the F_2 seemed to be that of a simple monohybrid. The data obtained are compared with the theoretical expected in Table V.

The ratio obtained for awn development is a good fit to the theoretical expected, as is shown by the high P value of .6065. The *fatua* parent contains a factor, or factors, for awn development which is intermediate in the heterozygote.

TABLE V

SEGREGATION FOR AWNS IN F_2 OF A CROSS BETWEEN *A. sativa* AND *A. fatua*, AND COMPARISON WITH A THEORETICAL 1 : 2 : 1 RATIO BY THE χ^2 METHOD

Class	F_2 frequencies		O−C	$(O-C)^2$	$\frac{(O-C)^2}{C}$
	Observed	Calculated			
Awnless	16	17.25	1.25	1.56	0.090
Intermediate	37	34.50	2.50	6.25	0.181
Awned	16	17.25	1.25	1.56	0.090
Total	69	69.00			$\chi^2 = 0.361$

P = <0.61

Articulation

The basal articulation of the grains on the F_1 plants was intermediate and tended to resemble the *sativa* rather than the *fatua* type. It approached the cultivated parent also in resistance to shattering. In the F_2, the plants were classified in three articulation groups, namely, *sativa*, intermediate, and *fatua*, according to whether the articulation of the kernels was similar to that of the parents, or intermediate. On this basis of classification the segregation in the F_2 failed to show a close fit to a theoretical 1 : 2 : 1 ratio. The data obtained are given in Table VI.

TABLE VI

SEGREGATION FOR BASAL ARTICULATION OF THE KERNELS IN THE F_2 FROM A CROSS BETWEEN *A. sativa* AND *A. fatua*, AND COMPARISON WITH A THEORETICAL 1 : 2 : 1 RATIO BY THE χ^2 METHOD

Class	F_2 frequencies		O−C	$(O-C)^2$	$\frac{(O-C)^2}{C}$
	Observed	Calculated			
sativa	25	17.25	7.75	60.06	3.482
Intermediate	28	34.50	6.50	42.25	1.225
fatua	16	17.25	1.25	1.56	0.090
Total	69	69.00			$\chi^2 = 4.797$

P = 0.0929

Delayed Germination

The results obtained from the study of delayed germination in the F_3 indicated that, after a period of approximately nine months of after-ripening, there was only one hybrid line out of 69 that appeared to show a distinctly low germinability. The inheritance of delayed germination in hybrids from *A. fatua* and *A. sativa* is being studied further by Johnson (18).

Linkages

One of the most striking features of the segregation in the F_2 and F_3 was the close linkage existing between articulation, awn development, rachilla

pubescence and callus pubescence—the characters of the fatuoid complex. Data compiled from independent genetic studies of each of these characters do not serve to show the relationships in the inheritance of these characters. An examination of the detailed data given in Table X, however, will serve to demonstrate this fact.

Among the *sativa* segregates, the absence of pubescence and of awn development, the *sativa* parental condition, greatly predominated; but there were a number of instances where these segregates developed callus pubescence or awns or both, usually to only a slight or moderate degree. It is not believed that this apparent break-up of the linkage relationship is caused by crossing over, for in no case were *fatua* and *sativa* expressions associated. It seems probable that relatively weak genes, in addition to and independent of the factors of the linkage group, give rise to these expressions. It is also possible that certain of these types may be due to extreme environmental influences.

Linkage between articulation, awn development and pubescence of rachilla and callus in crosses between *A. fatua* and *A. sativa* has been reported by a number of investigators. The results of Surface (27) and Fedorova (6) agree in general with the present data. The reader is referred to Florell (7) for a review of the literature on the genetics of floret articulation, and to Johnson (17) for a review of the literature on the genetics of awn development and of rachilla pubescence.

Lemma color was inherited independently of the characters, awn development, articulation, rachilla pubescence and callus pubescence. The literature on the genetics of lemma color has recently been reviewed by Johnson (17).

It was found that lemma pubescence was never expressed on white grains, and that in all but four F_2 plants (See Table X, Plants 50-3, 8, 12 and 51-11, all black *sativa, Bw*) it was expressed on black grains. All four of these plants produced some progeny with lemma pubescence. Obviously either lemma pubescence is linked with black lemma color, or it is inhibited by the factors for white lemma color. Surface (27) and Florell (7) observed a very small proportion of black grains with glabrous lemmas. Surface also observed a few non-black grains with pubescent lemmas. Both of these workers assumed that a condition of almost complete linkage existed between the factor for black lemma color and the factor for lemma pubescence. Love and Craig (20) found complete correlation between black color and pubescence, and between yellow color and glabrousness of the lemma. They concluded that the factor for yellow color inhibited the expression of the factor for lemma pubescence. Philp (24) obtained results in crosses between *A. fatua* and *A. sativa* which indicated the presence of two factors for lemma pubescence, one linked with, and one independent of, the factor for black lemma. The factors for white lemma (the double recessive) inhibited lemma pubescence.

An inspection of the data in Table X will provide more detailed information on the characteristics of the individual F_2 plants and their breeding behavior in the F_3.

Factorial Statement of Inheritance of Differential Characters

After each character had been studied for its individual mode of inheritance, and for its genetic relationship to the other characters, the task of devising a single scheme of inheritance for the entire group of characters was undertaken. The preliminary studies on individual characters disclosed the fact that close linkage existed between the characters,—articulation, awn development, rachilla pubescence and callus pubescence. As each of these characters was essentially monogenic in inheritance, it is assumed that in this material the linkage group is controlled by a single complex gene, or a closely linked group of genes. Specimens have been observed in the material with a complexity of characters, which indicate that the gene complex is not always transmitted as a unit. Jones (19) points out "that mutations have occurred in the C chromosome showing different degrees of complexity; fatuoids, sub-fatuoids, semi-fatuoids and mere strongly awned types, indicating that the genes mutate independently". The preliminary studies disclosed the fact that lemma color was controlled by two factors which were completely independent of the gene complex. The factor for lemma pubescence appeared to be linked with the factor for black lemma color. Considering these facts together it may be assumed that the inheritance of all characters in question is determined, essentially, by three factors,—the gene complex plus the two factors for color.

The probable presence of factors for awn development and callus pubescence, in addition to the gene complex, has been disregarded for two main reasons: first, because the expressions of these genes were in all cases relatively weak and of little significance to the objective of the investigation; second, it is convenient and desirable to explain the mode of inheritance and character relationships as simply as its essential nature will permit.

On the basis of the factorial interactions several phenotypes are to be expected. In the first place, the gene complex will determine the general types, *fatua*, intermediate, and *sativa*, which are based primarily on type of articulation. Secondly, the independent factors for lemma color will produce black, gray or white forms in each of these general types. Thus, the following phenotypes may be expected: black, gray, and white, *fatua* types; black, gray, and white, intermediate types; black, gray, and white, *sativa* types.

Based on observed data, the following factorial interactions and representations are assumed:

B—represents an epistatic factor for black lemma color.

G—represents a factor for gray lemma color; it is epistatic to *b* or *g* or both, but hypostatic to *B*.

W—represents a gene complex controlling awn development, articulation and rachilla and callus pubescence; *WW* produces *fatua*, *Ww* intermediate, and *ww sativa* types.

The following symbols are given to the various expected phenotypes:

BW, black *fatua*	BI, black intermediate	Bw, black *sativa*
GW, gray *fatua*	GI, gray intermediate	Gw, gray *sativa*
bW, white *fatua*	bI, white intermediate	bw, white *sativa*

A hypothetical inheritance scheme may now be stated as follows:

P_1 — *BBWWGG* × *bbwwgg* (BW) (bw)

F_1 — *BbWwGg* (BI)

F_2		*Genotype*	*Phenotype*	F_3 *behavior*
27	1	*BBGGWW*	BW	Homozygous BW
	2	*BbGGWW*	BW	3BW : 1GW
	2	*BBGGWw*	BI	1BW : 2BI : 1Bw
	2	*BBGgWW*	BW	Homozygous BW
	4	*BbGGWw*	BI	3BW : 6BI : 3Bw : 1GW : 2GI : 1Gw
	4	*BBGgWw*	BI	1BW : 2BI : 1Bw
	4	*BbGgWW*	BW	12BW : 3GW : 1bW
	8	*BbGgWw*	BI	F_2 segregation repeated 12BW : 24BI : 12Bw : 3GW : 6GI : 3Gw : 1bW : 2bI : 1bw
9	1	*BBggWW*	BW	Homozygous BW
	2	*BbggWW*	BW	3BW : 1bW
	2	*BBggWw*	BI	1BW : 2BI : 1Bw
	4	*BbggWw*	BI	3BW : 6BI : 3Bw : 1bW : 2bI : 1bw
9	1	*BBGGww*	Bw	Homozygous Bw
	2	*BbGGww*	Bw	3Bw : 1Gw
	2	*BBGgww*	Bw	Homozygous Bw
	4	*BbGgww*	Bw	12Bw : 3Gw : 1bw
9	1	*bbGGWW*	GW	Homozygous GW
	2	*bbGGWw*	GI	1GW : 2GI : 1Gw
	2	*bbGgWW*	GW	3GW : 1bW
	4	*bbGgWw*	GI	3GW : 6GI : 3Gw : 1bW : 2bI : 1bw
3	1	*BBggww*	Bw	Homozygous Bw
	2	*Bbggww*	Bw	3Bw : 1bw
3	1	*bbGGww*	Gw	Homozygous Gw
	2	*bbGgww*	Gw	3Gw : 1bw
3	1	*bbggWW*	bW	Homozygous bW
	2	*bbggWw*	bI	1bW : 2bI : 1bw
	1	*bbggww*	bw	Homozygous bw

The actual F_2 data conform fairly well with the theoretical expected calculated on the basis of the preceding hypothesis. The χ^2 test for the goodness of fit of observed and calculated ratios is given in Table VII.

The resulting P value of 0.2547 indicates fair conformity between these series of frequencies. Approximately 50% of the χ^2 value is due to the excessive number of homozygous black cultivated types (Bw) and homozygous gray types (Gw).

TABLE VII

SEGREGATION FOR LEMMA COLOR AND WILD-COMPLEX IN THE F_2 FROM A CROSS BETWEEN *A. sativa* AND *A. fatua*, AND COMPARISON WITH A THEORETICAL 12 : 24 : 12 : 3 : 6 : 3 : 1 : 2 : 1 RATIO BY THE χ^2 METHOD

Phenotype		F_2 frequencies		O−C	$(O-C)^2$	$\frac{(O-C)^2}{C}$
		Observed	Calculated			
Black, wild	*BW	14	12.94	1.04	1.08	0.083
Black, intermediate	BI	21	25.87	4.87	23.72	0.917
Black, cultivated	Bw	19	12.94	6.06	36.72	2.838
Gray, wild	GW	1	3.23	2.23	4.97	1.539
Gray, intermediate	GI	4	6.47	2.47	6.10	0.943
Gray, cultivated	Gw	6	3.23	2.77	7.67	2.375
White, wild	bW	1	1.08	.08	.06	0.056
White, intermediate	bI	3	2.16	.84	.71	0.329
White, cultivated	bw	0	1.08	1.08	1.17	1.083
Total		69	69.00			$\chi^2 = 10.163$

P = 0.2547

* *See p.* 716 *for legend of symbols used.*

As a further check on the validity of the hypothesis, a comparison was made between theoretical and actual frequencies of occurrence of F_2 genotypes. The genotypes of F_2 individuals were, with few exceptions, determinable through observation of the individual's breeding behavior in the F_3. Certain groupings of genotypes were necessary owing to inability to determine the specific genotypes, either because of the epistasis of factor *B* in black phenotypes, or because of environmental conditions which prevented distinction between gray and white phenotypes in the F_3. The actual genotypic frequencies, determined from the F_3, are given in Table X. The fact that it was possible to determine the F_2 genotypes from F_3 data, and the fact that there is such close general correspondence between actual and expected frequencies of these genotypes, give strong support to the validity of the hypothesis.

The appearance of three heterozygous fatuoid types (bI) and one homozygous fatuoid type (bW) as Mendelian segregates from this artificial cross, is especially interesting since it furnishes definite proof that these types can originate as a result of hybridization between *A. sativa* and *A. fatua.* Their further appearance in the F_3 as Mendelian segregates from such F_2 plants as *BbWw* and *BbWW* makes the proof rather conclusive. In morphological characteristics and in breeding behavior these "synthetic" fatuoid types correspond exactly to the common fatuoid described in the introduction to this paper and illustrated in Plate 2, *C.*

Natural Hybrids

The occurrence of natural crossing between *A. sativa* and *A. fatua* has been proved beyond any doubt. Coffman and Wiebe (4) observed several instances of natural crossing between *A. fatua* and *A. sativa* at Aberdeen, Idaho. Derick

(5) found 0.1% of natural crossing between these species at Ottawa, Canada. Crépin (1) found a plant in a field of oats in France which was shown by breeding tests to have had its origin through a natural cross between *A. fatua* and *A. sativa*. Natural crossing between varieties of *A. sativa* has been shown to vary in frequency of occurrence from a small fraction of one per cent to several per cent. The literature on this subject has been reviewed at considerable length by Stanton and Coffman (25), and more recently by Harrington (11).

A study of the amount of natural crossing that may take place between *A. sativa* and *A. fatua* when grown in adjacent rows has been made by Johnson (18) as part of a study complementary to the investigation reported herein. He found 0.362% of natural hybrids in *A. fatua* when grown adjacent to Victory (*A. sativa*), and 0.056% natural hybrids in the Victory.

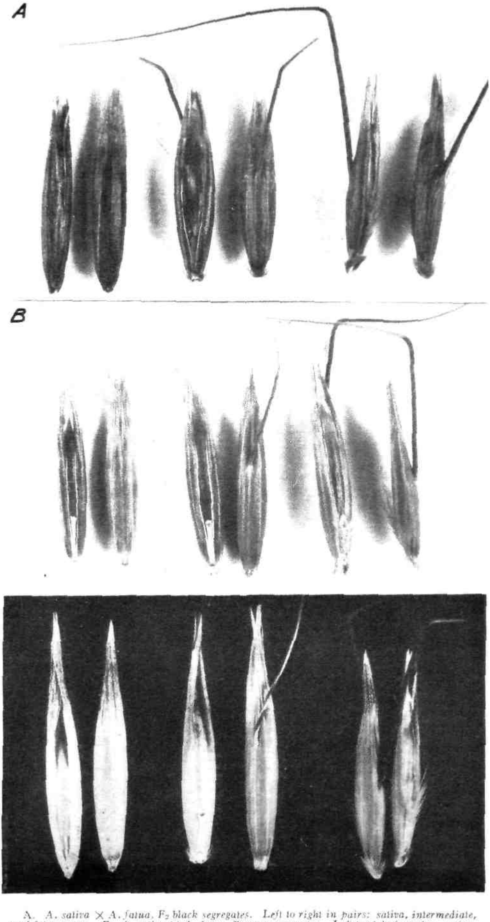
As indicated earlier in this paper, aberrant types that appeared to be segregates from natural crosses between *A. sativa* and *A. fatua* were found in seed stocks and fields of oats. Many of these were grown and studied in the same way as the segregates from the artificial crosses between these same species. Some of the natural hybrids proved to be homozygous, while others were heterozygous. All of the latter group produced segregating populations which corresponded more or less closely to certain types of segregation observed in the F_3 from the artificial cross.

Plants from 41 seeds from a black aberrant of the BI type, found in Victory oats at the University of Alberta, Edmonton, segregated in the following generation with a ratio of 32 black (B) to 9 non-black (G and b). The numbers were too small in the recessive group to expect a good ratio, and consequently they were combined. The ratio of 32 black to 9 non-black is a good fit to the theoretical expected 3 : 1 ratio with odds of less than 1 : 1 against the occurrence of a deviation as great as, or greater than this one, on the basis of random sampling. The complete data on the breeding behavior of this line are given in Table VIII, together with the theoretical expected based on the segregation of similar types in the F_3 from the artificial cross.

The test of goodness of fit by the χ^2 method shows a fair agreement between the observed and calculated frequencies, with a P value of 0.3268. Nine of the plants were typical wild oats (BW), three were typical homozygous fatuoids (bW), and four were typical heterozygous fatuoids (bI).

Thirty-nine plants were grown from a grayish aberrant of the GI type obtained from a field of Banner at Wetaskiwin, Alberta. This line segregated for gray and white and the gene complex. The data on the breeding behavior are given in Table IX, together with the theoretical expected based on the segregation of similar types in the F_3 from the artificial cross.

The test of goodness of fit by the χ^2 method shows an excellent agreement between the observed and calculated frequencies, the P value being 0.8622. Five of the plants were gray wilds (GW), two were typical homozygous fatuoids (bW), and four were typical heterozygous fatuoids (bI).



A. *A. sativa* × *A. fatua*, F_2 black segregates. *Left to right in pairs: sativa, intermediate, and fatua types.* B. *A. sativa* × *A. fatua*, F_2 gray segregates. *Left to right in pairs: sativa, intermediate, and fatua types.* C. *A. sativa* × *A. fatua*, F_2 white segregates. *Left to right in pairs: sativa, intermediate, and fatua types.*

TABLE VIII

BREEDING BEHAVIOR OF AN *A. sativa* × *A. fatua* NATURAL HYBRID AND COMPARISON OF OBSERVED AND THEORETICAL RATIOS BY THE χ^2 METHOD

Phenotype	F_2 frequencies		O−C	$(O-C)^2$	$\frac{(O-C)^2}{C}$
	Observed	Calculated			
BW	9	7.7	1.3	1.69	0.219
BI	17	15.3	1.6	2.56	0.166
Bw	6	7.7	1.7	2.89	0.375
GW and bW	2 + 3	2.6	2.4	5.76	2.215
GI and bI	0 + 4	5.1	1.1	1.21	0.237
Gw and bw	0	2.6	2.6	6.76	2.600
Total	41	41.0			$\chi^2 = 5.812$

P = 0.3268

TABLE IX

BREEDING BEHAVIOR OF AN *A. sativa* × *A. fatua* NATURAL HYBRID AND COMPARISON OF OBSERVED AND THEORETICAL RATIOS BY THE χ^2 METHOD

Phenotype	F_2 frequencies		O−C	$(O-C)^2$	$\frac{(O-C)^2}{C}$
	Observed	Calculated			
GW	5	7.31	2.31	5.34	0.731
GI	18	14.63	3.37	11.36	0.776
Gw	8	7.31	0.69	0.48	0.066
bW	2	2.44	0.44	0.19	0.078
bI	4	4.87	0.87	0.76	0.156
bw	2	2.44	0.44	0.19	0.078
Total	39	39.00			$\chi^2 = 1.885$

P = 0.8622

Many other examples could be given of aberrant types, or natural hybrids from *A. sativa* × *A. fatua* crosses, that give rise to fatuoid types which are morphologically identical to heterozygous and homozygous fatuoids. The above examples are sufficient to demonstrate that some of the aberrant types originated from natural crosses between *A. fatua* and *A. sativa*, and that certain segregates from these natural hybrids are typical fatuoids.

TABLE X

DESCRIPTION OF PARENT AND INDIVIDUAL F_2 PLANTS FROM CROSSES BETWEEN *A. fatua* AND *A. sativa*, AND THEIR BREEDING BEHAVIOR IN THE F_3*

F_2 plant No.	Description of F_2 plants								F_3 breeding behavior	
	Lemma color	Awn	Pubescence			Articulation	Phenotype	Genotype	Expected ratio	Observed data
			Rachilla	Callus	Lemma					
Wild	Black	VSt_3	VL_3	L_3	L_3	aa_3	BW	*BBGGWW*	Homozygous BW	Homozygous BW
Sel. 76	White	0	0	0	0	AA_1	bw	*bbggww*	Homozygous bw	Homozygous bw
49-1	Black	VSt_3	VL_3	L_3	L_{2+}	aa_3	BW	*BB - - WW*	Homozygous BW	27BW
2	White	I_1-	0	M_2	0	AA_2	bI	*bbggWw*	1bW : 2bI : 1bw	6bW : 17bI : 5bw
3	Brown to black	VSt_3	VL_3	L_3	L_3	aa_3	BW	*BbggWW*	3BW : 1bW	19BW : 6bW (1B1)†
4	Brown to black	ISt_1	Sh_1	M_{2+}	VL_3	AA_2	BI	*BB - - Ww*	1BW : 2BI : 1Bw	4BW : *15BI* : 4Bw
5	Brown to black	VSt_3	VL_3	L_3	L_{2+}	aa_3	BW	*BbggWW*	3BW : 1bW	20BW : 4bW
6	Gray	VSt_3	VL_3	L_{2+}	0	aa_3	GW	*bbGgWW*	3GW : 1bW	6GW : 17bW
7	*Brown to black*	ISt_{1+}	0	L_2	M_1	AA_2	BI	*BbggWw*	3BW : 6BI : 3Bw : 1bW : 2bI : 1bw	3BW : 6BI : 3Bw : 1bW : 1bw
8	Brown to black	0	0	0	M_2	AA_1	Bw	*BB - - ww*	Homozygous Bw	17Bw
9	Brown to black	Wk-St_2	0	Sh_2	M_2	AA_2	BI	*BbGgWw*	F_2 ratio*	2BW : 12BI : 7Bw : 1GW : 1GI : 1Gw : 3bW : 2BI
10	Brown to black	ISt_1	Sh_{1+}	M_2-	L_{2+}	AA_2	BI	*BbggWw*	3BW : 6BI : 3Bw : 1bW : 2bI : 1bw	4BW : 9BI : 4Bw : 1bW : 3bI : 1bw
12	Dark brown	ISt_1	0	M_{2+}	M_{2+}	AA_2	BI	*BbggWw*	3BW : 6BI : 3Bw : 1bW : 2bI : 1bw	3BW : 12BI : 4Bw : 2bW : 2bI
13	Brown to black	ISt_1-	0	M_{2+}	Sh_{1+}	AA_2	BI	*BbggWw*	3BW : 6BI : 3Bw : 1bW : 2bI : 1bw	3BW : 6BI : 5Bw : 2bW : 3bI : 1bw
14	Brown to black	Wk_{1+} (long)	0	M_2	L_2	AA_3	BI	*BbGgWw*	F_2 ratio	2BW : 10BI : 6Bw : 2GI : 1bW : 1bI
15	Brown to black	ISt_1-	0	M_2	M_1	AA_{2-3}	BI	*BbggWw*	3BW : 6BI : 3Bw : 1bW : 2bI : 1bw	2BW : 9BI : 6Bw : 6bI : 1bw
50-1	Brown to black	VSt_3	VL_3	L_3	VL_3	aa_3	BW	*BbggWW*	3BW : 1bW	14BW : 8bW
2	Brown to black	0	0	0	Sh_1-	AA_1	Bw	*Bbggww*	3Bw : 1bw	15Bw : 8bw
3	Brown to black	ISt_2	0	M_{2+}	0	AA_2	BI	*BbggWw*	3BW : 6BI : 3Bw : 1bW : 2bI : 1bw	5BW : 9BI : 6Bw : 1bW : 2bI : 1bw
4	Brown to black	ISt_1	Sh_1-	L_{2+}	M_2	AA_2	BI	*BbggWw*	3BW : 6BI : 3Bw : 1bW : 2bI : 1bw	4BW : 6BI : 3Bw : 1bW : 2b –

* *F_2 ratio*—12BW : 24BI : 12Bw : 3GW : 6GI : 3Gw : 1bW : 2bI : 1bw.
† *Bracketed observed data are obvious mixtures or environmental variations.*
See page 723 for explanation of symbols and subscripts.

TABLE X—*Continued*

DESCRIPTION OF PARENT AND INDIVIDUAL F_2 PLANTS FROM CROSSES BETWEEN *A. fatua* AND *A. sativa*, AND THEIR BREEDING BEHAVIOR IN THE F_3*

F_2 plant No.	Description of F_2 plants								F_3 breeding behavior	
	Lemma color	Awn	Pubescence			Articulation	Phenotype	Genotype	Expected ratio	Observed data
			Rachilla	Callus	Lemma					
5	Brown to black	St_3–	Sh_1–	M_1	L_3	AA_2	Bl	*BbGGWw*	3BW : 6Bl : 3Bw : 1GW : 2Gl : 1Gw	*5BW : 5Bl : 1Gl*
6	Brown to black	Wk_1–	Sh_1–	M_2	M_1–	AA_{1-2}	Bl	*BbggWw*	3BW : 6Bl : 3Bw : 1bW : 2bl : 1bw	5BW : 8Bl : 5Bw : 3bW : 1bl
7	Brown to black	VSt_3	VL_3	L_3	L_{2+}	aa_3	BW	*BbggWW*	3BW : 1bW	19BW : 4bW
8	Brown to black	0	0	0	0	AA_1	Bw	*BbGgww*	12Bw : 3Gw : 1bw	10Bw : 2Gw : 1bw
9	Brown to black	VSt_3	VL_3	L_3	L_{2+}	aa_3	BW	*BbggWW*	3BW : 1bW	20BW : 5bW
10	Brown to black	0	0	0	M_2	AA_1	Bw	*BB - - ww*	Homozygous Bw	29Bw
11	Brown to black	VSt_3	VL_3	L_3	L_{2+}	aa_3	BW	*BbggWW*	3BW : 1bW	15BW : 4bW
12	Brown to black	Wk_1	0	M_2	0	AA_{1-2}	Bw	*BbGgww*	12Bw : 3Gw : 1bw	18Bw : 4Gw : 4bw
13	Brown to black	St_2	Sh_1–	M_2	M_2	AA_{1-2}	Bl	*BbGgWw*	F_2 ratio	4BW : 8Bl : 4Bw : 1Gl : 1Gw : 3bW : 1bl
14	Brown to black	0	0	0	M_3	AA_{1-2}	Bw	*BB - - ww*	Homozygous Bw	24Bw (1bw)
15	Gray	0	0	0	0	AA_{1-2}	Gw	*bbGgww*	3Gw : 1bw	Immature Gw and bw
16	Black	VSt_3	VL_3	L_3	L_3	aa_3	BW	*BB - - WW*	Homozygous BW	14BW (1bW : 2bw)
51-1	Black	VSt_3	VL_3	L_3	L_3	aa_3	BW	*BbggWW*	3BW : 1bW	*10BW : 2bW*
2	Brown to black	St_3	Sh_1	L_3–	M_1	AA_3	Bl	*BbggWw*	3BW : 6Bl : 3Bw : 1bW : 2bl : 1bw	5BW : 9Bl : 7Bw : 2bW : 5bl : 1bw
3	Brown to black	St_3	0	0	L_2	AA_{1-2}	Bw	*BB - - ww*	*Homozygous Bw*	23Bw
4	Black	VSt_3	VL_3	L_3	L_3	aa_3	BW	*BB - - WW*	Homozygous BW	28BW (Bw)
5	Gray-brown	St_3	0	M_1–	0	AA_1	Gw	*bbGGww*	Homozygous Gw	25Gw
6	Brown to black	0(VSt)	0	M_1	M_1	AA_2	Bw	*Bbggww*	3Bw : 1bw	18Bw : 4bw
7	Gray-brown to black	St_3	0	Sh-M_{1+}	M_1	AA_2	Bl	*BbggWw*	3BW : 6Bl : 3Bw : 1bW : 2bl : 1bw :	9BW : 5Bl : 4Bw : 2bW : 1bl : 2bw
8	Brown to black	St_3	0	M_2–	M_1	AA_3	Bl	*BbggWw*	3BW : 6Bl : 3Bw : 1bW : 2bl : 1bw	2BW : 5Bl : 2Bw : 2bl : 1bw
9	Brown to black	St_3	0	M_2	L_3	AA_3	Bl	*BB - - Ww*	1BW : 2Bl : 1Bw	4BW : 12Bl : 8Bw
10	Black	VSt_3	VL_3	L_3	L_3	aa_3	BW	*BbggWW*	3BW : 1bW	15BW : 5bW

* *F_2 ratio*—12BW : 24Bl : 12Bw : 3GW : 6Gl : 3Gw : 1bW : 2bl : 1bw.
Bracketed observed data are obvious mixtures or environmental variations.

TABLE X—*Continued*

DESCRIPTION OF PARENT AND INDIVIDUAL F_2 PLANTS FROM CROSSES BETWEEN *A. fatua* AND *A. sativa*, AND THEIR BREEDING BEHAVIOR IN THE F_3*

F_2 plant No.	Description of F_2 plants								F_3 breeding behavior	
	Lemma color	Awn	Pubescence			Articu-lation	Pheno-type	Genotype	Expected ratio	Observed data
			Rachilla	Callus	Lemma					
11	Brown to black	I_1	0	0	0	AA_1	Bw	*Bbggww*	3Bw : 1bw	17Bw : 7bw
52-1	Gray	Wk_1–	0	M_1–	0	AA_1	Gw	*bbGgww*	3Gw : 1bw	7Gw : 1bw
2	Cream	I_1+	0	M_2	0	AA_{2-3}	bI	*bbggWw*	1bW : 2bI : 1bw	2bW : 10bI : 7bw
3	Brown to black	ISt_1+	0	L_3	M_2	AA_{2-3}	BI	*BB - - Ww*	1BW : 2BI : 1Bw	10BW : 15BI : 5Bw
4	Brown to dark brown	Wk_1–	0	M_2+	M_2	AA_{2-3}	BI	*BbGgWw*	F_2 ratio	7BW : 10BI : 6Bw : 1GW : 1GI : 1bW
5	Gray	I_1	0	M_2	0	AA_2	GI	*bbGgWw*	3GW : 6GI : 3Gw : 1bW : 2bI : 1bw	1GW : 9GI : 6Gw : 4bW : 4bI : 4bw
6	Cream	VSt_2	VL_3	L_2	0	aa_3	bW	*bbggWW*	Homozygous bW	26bW
7	Brown to black	0	0	0	L_2	AA_{1-2}	Bw	*BB - - ww*	Homozygous Bw	20Bw
8	Gray	St_2+	0	M_2+	0	AA_{2-3}	GI	*bbGgWw*	3GW : 6GI : 3Gw : 1bW : 2bI : 1bw	3GW : 4GI : 5Gw : 3bW : 4bI : 5bw
9	Brown to black	0	0	0	M_2	AA_1	Bw	*BB - - ww*	Homozygous Bw	19Bw
10	Brown to black	0	0	Sh_1	L_3	AA_{1-2}	Bw	*BB - - ww*	Homozygous Bw	20Bw
11	Brown to black	VSt_3	VL_3	L_2	L_3	aa_3	BW	*BbggWW*	3BW : 1bW	15BW : 5bW (1BI : 1bI)
12	Brown to black	Wk_1	0	0	M_2	AA_1	Bw	*BB - - ww*	Homozygous Bw	30Bw
13	Brown to black	VSt_3	VL_3	L_3	L_4	aa_2	BW	*BbggWW*	3BW : 1bW	12BW : 11bW
14	Brown to dark brown	ISt_2	0	M_2	Sh_1–	AA_2	BI	*BbggWw*	3BW : 6BI : 3Bw : 1bW : 2bI : 1bw	4BW : 11BI : 4Bw : 1bW : 2bI : 3bw
15	Cream	I_1	0	M-L_2–	0	AA_{2-3}	GI	*bbGgWw*	3GW : 6GI : 3Gw : 1bW : 2bI : 1bw	1GW : 7GI : 4Gw : 3bW : 4bI : 1bw
16	Brown to black	Wk_1	0	0	L_3	AA_{1-2}	Bw	*BB - - ww*	Homozygous Bw	28Bw
17	Gray	0	0	0	0	AA_1	Gw	*bbGgww*	3Gw : 1bw	10Gw : 3bw
18	Brown to black	VSt_3	VL_3	L_3	L_3	aa_3	BW	*BB - - WW*	Homozygous BW	23BW (3BI)
19	Brown to black	Wk_1–	0	Sh_1–	M_1	AA_{1-2}	Bw	*BbGGww*	3Bw : 1Gw	18Bw : 8Gw
20	Brown to black	0	0	0	M_2	AA_1	Bw	*BB - - ww*	Homozygous Bw	25Bw
21	Brown to black	0	0	0	L_2	AA_{1-2}	Bw	*BB - - ww*	Homozygous Bw	24Bw
22	Gray	Wk_1	0	M_2+	0	AA_{2-3}	Gw	*bbGgww*	3Gw : 1bw	9Gw : 15bw

* *F_2 ratio*—12BW : 24BI : 12Bw : 3GW : 6GI : 3Gw : 1bW : 2bI : 1bw.
Bracketed observed data are obvious mixtures or environmental variations.

TABLE X—*Concluded*

DESCRIPTION OF PARENT AND INDIVIDUAL F_2 PLANTS FROM CROSSES BETWEEN *A. fatua* AND *A. sativa*, AND THEIR BREEDING BEHAVIOR IN THE F_3

F_2 plant No.	Description of F_2 plants								F_3 breeding behavior	
	Lemma color	Awn	Pubescence			Articu-lation	Pheno-type	Genotype	Expected ratio	Observed data
			Rachilla	Callus	Lemma					
23	Gray	Wk$_1$	0	M-L$_{2+}$	0	AA$_2$	GI	*bbGgWw*	3GW : 6GI : 3Gw : 1bW : 2bI : 1bw	1GW : 5GI : 7Gw : 1bw
24	Brown to black	I$_2$	0	Sh$_3$	Sh$_1$	AA$_3$	BI	*BbGGWw*	3BW : 6BI : 3Bw : 1GW : 2GI : 1Gw	3BW : 7BI : 3Bw : 1GW : 4GI : 4Gw (2bW)
25	Cream	Wk$_{1-}$	0	M-L$_{1+}$	0	AA$_3$	bI	*bbggWw*	1bW : 2bI : 1bw	3bW : 17bI : 6bw (4Bw)
26	Brown to black	0	0	Sh$_{1-}$	Sh$_{1-}$	AA$_1$	Bw	*BbGGww*	3Bw : 1Gw	11Bw : 5Gw
27	Cream	0	0	0	0	AA$_1$	Gw	*bbGGww*	Homozygous Gw	24Gw (3bw)
28	Brown to black	0	0	0	L$_3$	AA$_{1-2}$	Bw	*BB - - ww*	Homozygous Bw	21Bw

Explanation of symbols and subscripts used in describing grain characters:

Awns

VSt—*very strong*, St—*strong*, ISt—*intermediate-strong*, I—*intermediate*, Wk—*weak*, O—*absent*.

Articulation

aa—*wild type*, AA—*semi-solidified type*.

Pubescence

VL—*very long*, L—*long*, M—*medium length*, Sh—*short*, O—*absent*.

Phenotype—Lemma color

B—*black*, G—*gray*, b—*white*.

Phenotype—for expression of the fatuoid complex of characters.

W—*wild*, I—*intermediate*, w—*cultivated*.

Numerical subscripts to all symbols denote frequency, or degree, of the character expression on a scale ranging as follows: 0, 1−, 1+, 2−, 2, 2+, 3− to 3. Thus L$_3$ *describes the condition of many long hairs, and* S$_1$ *the condition of few short hairs.*

In the case of awns, the subscript $_3$ *indicates the presence of awns on every spikelet of the panicle; in the case of pubescence, it indicates, approximately, the maximum hairiness of the character in question to be observed in the material; in the case of articulation it indicates large size of attachment.*

Bracketed observed data are obvious mixtures or environmental variations.

General Discussion and Conclusions

It has been demonstrated that there are two grain types occurring in the segregation from *A. fatua* × *A. sativa* crosses, which are morphologically and genetically identical with heterozygous and homozygous fatuoids, respectively.

The opponents of the natural crossing hypothesis may contend that these grain types are not true segregates from the cross, but that they have originated through chromosome aberration as a result of chromosomal incompatibilities introduced by the interspecific nature of the crosses. Huskins (16) has recently stated that "the origin of fatuoids or speltoids from crosses of *A. sativa* and *T. vulgare* with other species of oats or wheat, respectively, is of course expected on the chromosome aberration explanation, especially since hybridization increases the frequency of irregular pairing. But the origin of fatuoids in the progeny of intervarietal or interspecific crosses by no means supports the earlier hypothesis of Tschermak and others, that crossing between *A. sativa* and *A. fatua* is *the* cause of their origin. It is rather an incidental issue that they sometimes arise from such crosses". Close study of the results of the present investigation will show that such a contention is untenable. The presence of a single genetic factor for articulation; the existence of a very close linkage relationship between the factor for articulation and the factors for awn development, rachilla pubescence and callus pubescence; and the presence of two factors for lemma color which are independent of the linkage group: these facts make segregation for the two fatuoid types in *A. sativa* × *A. fatua* crosses inevitable. That the mode of inheritance of each of these characters is according to Mendelian laws, when treated independently, strengthens the conclusion that the inheritance of the gene complex is also according to Mendelian laws. Huskins (14) states that "In general it seems safe to assume that all intermediate forms which on selfing give complex segregation involving a number of characters, have arisen from the crossing of *A. sativa* and *A. fatua*". We are in complete agreement with this opinion. When fatuoids appear as segregates from such hybrid forms in normal genetic ratios, there can be no doubt that they originate through the process of hybridization and recombination. The point which really needs to be explained is not the appearance of the fatuoid, but the more or less complete selective elimination of segregates other than fatuoids.

In the material investigated the fatuoid types occurred with relative rarity in the segregating population; the various black and gray types were far more numerous. How is it then that fatuoids are not found invariably in association with a host of black and gray off-types? The answer is that fatuoids have been found with considerable regularity in association with these other aberrants. This is particularly true in fields grown from seed stocks which have not undergone selection for a considerable time. Field inspectors state that these types are of common occurrence throughout the country, particularly in districts where unselected seed is used. One inspector tells of observing many bushels of oats in which segregating types were as

numerous as the *sativa* types. A small percentage of the segregates were what are commonly called fatuoids. The University of Alberta, and several private seed growers, have found it necessary from time to time to remove black and gray off-types, in addition to fatuoids, from registered seed stocks. It is undoubtedly true, however, that in well-selected seed stocks, such as are found at experiment stations and on seed farms, fatuoids are usually found unassociated with other off-types. When selection is practised in a field or plot, the black and gray segregates from a natural cross would be readily observed and removed, the homozygous fatuoid types would also be removed, but the heterozygous fatuoid type, similar in color to the cultivated parent would, in many cases, escape notice and be propagated with the selected seed stock.

Garber (8) in disagreeing with Zade's statement that the single factor difference in heterozygous false wild oats could be accounted for by the elimination of other factors in earlier generations of the cross, states that "one must account for the selective elimination of all phenotypes except the three types, cultivated, intermediate and true false wild oats". Our observations in the field, and in the seed cleaning plants, have shown that the selective elimination of all phenotypes except the three types mentioned above, is exactly what one would expect to take place following hybridization of *A. fatua* with *A. sativa* in cultivated oat fields in which selection is practised. In rogueing in the field all the segregating types except the heterozygous fatuoid and the true *sativa* type are so distinctively off-type that they are readily recognized and eliminated. The same is true in the hand picking of pure seed stocks. This opinion is substantiated further by the fact, as stated by Huskins (14), that "In nearly all recorded cases the heterozygous form appears first." And the possibility of the fatuoid type being maintained to the exclusion of other segregating types is enhanced, as suggested by Garber (8), in that, "their further dissemination is facilitated by natural crossing".

The extent to which the heterozygous fatuoid will escape detection in a given variety, probably varies directly with the degree of awn development in that variety; for under field conditions the strong development of awns is the only feature by which the heterozygous fatuoid plant can readily be distinguished from the normal plant, hence if the normal plant is also strong-awned there will be little or no distinction. This theory is borne out by the fact that fatuoids have never been known to occur in Selection 76, which is entirely awnless, while they occur quite frequently in our common, awned varieties. All that has been said leads up to Zade's statement (30) that the heterozygous fatuoids are not F_1 types directly from a natural cross, but are segregate types from a cross which occurred several years before.

The writers are convinced that fatuoids arise through natural crossing between *A. fatua* and *A. sativa*. But, while they are certain that fatuoids originate from this cross, they also admit the possibility of origin in other ways. The incompatibility of chromosomes brought together in crosses between *A. fatua* and *A. sativa* may at times produce fatuoids through chromo-

somal aberration. Jones (19) as a result of his studies on this problem, does not question the validity of the chromosome aberration hypothesis, but does maintain that it is inapplicable to Huskins "A" series showing normal segregation and chromosome number. The present results are held to refute neither the chromosomal aberration theory, nor the gene mutation theory, regarding the origin of fatuoids, but they do support the hypothesis that fatuoids may originate also as normal Mendelian segregates from crosses between *A. fatua* and *A. sativa*.

Summary

1. Numerous aberrant types of oats, including fatuoids, intermediate between cultivated (*A. sativa*) and wild (*A. fatua*) oats were found in fields of oats and in various seed stocks.

2. It has been demonstrated that many of these aberrants originated through natural hybridization between cultivated and wild oats.

3. Artificial crosses were made between cultivated oats and wild oats. An array of types was produced in the F_2 and F_3 similar to the aberrants occurring naturally in the field.

4. Genetic studies demonstrated conclusively that these intermediate types were the result of normal Mendelian segregation.

5. Fatuoids, or false wild oats, identical morphologically and genetically with the common fatuoid, appeared as normal Mendelian segregates from both the artificial and natural crosses.

6. The non-fatuoid type segregates are eliminated more or less as a result of selection.

7. These results are not held to disprove the chromosome aberration or gene mutation theories of origin; but they are believed to establish definitely the fact that fatuoids may frequently originate through natural hybridization between *A. sativa* and *A. fatua*.

Acknowledgment

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A MATHEMATICAL THEORY OF THE GROWTH OF POPULATIONS OF THE FLOUR BEETLE, *TRIBOLIUM CONFUSUM*, DUV.

III. THE EFFECT UPON THE EARLY STAGES OF POPULATION GROWTH OF CHANGES IN THE NUTRITIVE VALUE, PALATABILITY AND DENSITY OF PACKING OF THE FLOUR MEDIUM[1]

BY JOHN STANLEY[2]

Abstract

The questions of the nutritive value of the flour and its palatability have been considered mathematically, and it is shown that the number of eggs present *at a time T, between the start of the culture and the time of hatching of the* first egg, is decreased when the flour is less nutritious or less palatable, or less tightly packed, and *vice versa*. It is further shown that the asymptotic value ξ toward which the egg population moves in the absence of hatching, is similarly affected.

The question of the mechanism underlying these effects is discussed.

Introduction

In a recent paper, Park (1) has shown from data experimentally obtained that the use of "conditioned" flour, *i.e.*, old flour which has been contaminated with frass, etc., by being used as a medium for growing *Tribolium* beetles for prolonged periods, results in fewer eggs in comparison with the numbers in cultures made up with fresh flour. Park indicates that this result may be due to several causes, *viz.*, the accumulation of excretory and other refuse products in the flour, the depletion with age of the food content of the populated flour and the possibility of increased cannibalistic egg-eating rates due to an effect of the conditioned flour on the beetles.

It is of interest to examine these results mathematically to see the underlying mechanism which brings about these changes.

Effects Due to Changes in the Nutritive Value and Palatability of the Flour

Effect upon the Number of Eggs Existing at a Time T

In a previous paper (2) the writer has shown that the number of eggs (N_2) existing in a *Tribolium* culture at a time T, $t_0 = t_2(1) < T < t_3(1)$, *i.e.*, during the interval from the start of the culture to the instant of hatching of the first egg to hatch is,

$$T = \frac{c}{a} N_2 + \frac{(ad - bc)}{a^2} \log_e \left(\frac{aN_2 + b}{b} \right) \tag{1}$$

where

$$a = R\epsilon N_{11} P_{11\cdot2} A_2 W_2 r^2_{11.2} - E_{11} P_{11\cdot2} r^2_{11.2} N_{11} = P_{11\cdot2} r^2_{11.2} N_{11} (R\epsilon A_2 W_2 - E_{11}) \ .$$
$$b = R\epsilon N_{11} P_{11\cdot1} A_1 W_1 K_{11} r^2_{11.1} G \ .$$
$$c = P_{11\cdot2} A_2 W_2 r^2_{11.2} \ .$$
$$d = P_{11\cdot1} A_1 W_1 K_{11} r^2_{11.1} G \ .$$

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and

R = sex ratio, *i.e.*, ratio of females to total.
ϵ = number of eggs laid per female per unit time.
N_{11} = number of adults at a time T.
N_2 = number of eggs at a time T.
$P_{11\cdot 2}$ = preference of adults for eggs as a food.
$P_{11\cdot 1}$ = a similar parameter for flour.
A_2 = percentage of assimilable nutrient material in eggs.
A_1 = a similar parameter for flour.
W_2 = weight of an egg.
W_1 = density of uncompressed flour.
$r_{11\cdot 2}$ = radius of perception of an adult for an egg.
$r_{11\cdot 1}$ = a similar parameter for flour.
G = volume of flour.
E_{11} = maintenance ration of adults, *i.e.*, the quantity of nutrient material which must be ingested per unit time by each adult to maintain the beetle's normal rate of metabolism and provide food for normal egg production.

From Equation (1) it can easily be shown that

$$\frac{\partial N_2}{\partial A_1} = \frac{\dfrac{(ad - bc)N_2}{A_1(aN_2 + b)} - \dfrac{(ad - bc)}{a^2 A_1} \log_e \left(\dfrac{aN_2 + b}{b}\right)}{\dfrac{c}{a} + \dfrac{ad - bc}{aN_2 + b}} \tag{2}$$

If, as is assumed is the case, $N_2 < -\frac{b}{a} = \xi$ where ξ is the asymptotic value towards which the egg population moves in the absence of hatching, $(ad-bc) < 0$, $\log_e \left(\frac{aN_2+b}{b}\right) < 0$ and $\frac{c}{a} < 0$. Hence all terms on the right of Equation (2) are negative and so the right side as a whole is positive, and $\frac{\partial N_2}{\partial A_1} > 0$.

From this it follows that if two cultures are started, one in old, non-nutritious flour, the other in good flour, the culture in the old flour will always contain less eggs, the assumed conditions of time and non-hatching being observed.*

Further, it is obvious that as $P_{11\cdot 1}$ is always paired with A_1 in the various equations, $\frac{\partial N_2}{\partial P_{11\cdot 1}} > 0$, and it is thus seen that the increased number of eggs in the culture made up with good flour results partly from protection afforded the eggs by reason of the greater nutritive value of the flour, and partly from protection resulting from greater *palatability* of the flour.

In connection with the differentiation by means of which Equation (2) was obtained, an interesting point arises. Calling the right side of the equation X, for the moment one is tempted to perform the differentiation in the usual way, and write, $\frac{\partial T}{\partial A_1} = \frac{\partial X}{\partial A_1}$ where $\frac{\partial T}{\partial A_1}$ is not necessarily equal to zero, by reason of the fact that as N_2 is a function of A_1 and T is a function of N_2, then T is also a function of A_1. However, one must realize that the variable T differs from the usual variable, such as the amount of gas in a liquid-gas

**As a matter of fact, it can be shown that the assumption as to non-hatching is unimportant, but the proof from the complicated equations obtaining when $T > t_h(1)$ is uninviting.*

system, subject to changes in pressure, etc. In the case under discussion, T may be thought of in two ways, first as a true variable, namely the "time at which a certain number of eggs, N_2, is found in the population" (N_2 being an ordinary variable assuming various values), and second, as true "Time", increasing steadily, and quite independent of A_1 or any other variable in the system.

As a matter of notation, T appears in the equations as if it were thought of in the first way, but if one sets up a series of populations with various constant values of A_1 and then examines them after the same time interval has passed in each case since their initiation, then T falls into the second class, as a completely independent variable, in particular, independent of A_1 and so $\frac{\partial T}{\partial A_1} \equiv 0$.

Critical Value of A_1

From (2, Equation 27, p. 648) we have,

$$N_2' = \frac{aN_2 + b}{cN_2 + d} \ . \tag{3}$$

Equation (3) may be written in the form

$$N_2' = \frac{\alpha + \beta A_1}{\gamma + \delta A_1} \ . \tag{4}$$

Clearly, then, when $N_2' = 0$. and the culture is at a standstill, $A_{1(0)}$ the critical value of A_1 is

$$A_{1(0)} = -\frac{\alpha}{\beta} = \frac{P_{11\cdot 2}\, r^2_{11\cdot 2}\, N_{11}(E_{11} - R\epsilon A_2 W_2)}{R\epsilon N_{11} P_{11\cdot 1} W_1 A_1 K_{11} r^2_{11\cdot 1} G} \ . \tag{5}$$

Where $(E_{11} - R\epsilon A_2 W_2)$ may be written as Ξ, this being the amount of nutrient material per unit time which must be ingested to maintain metabolism and movement, etc., apart from forming and laying eggs.

From this it follows that the greater the preference for eggs, the greater the number of adults, the greater the radius of perception for eggs, and the greater the amount of energy expended in movement, the more nutritious must be the flour, if the culture is to progress*.

Further, from an examination of the denominator, it is seen that the greater the sex ratio (ratio of females to total), the more eggs laid per female per day, the greater the preference for flour, the more densely the flour is packed, the more the flour will compress as the beetles move through it, the greater the radius of perception for flour, and the *larger the culture*, the more the flour can be allowed to deteriorate without bringing the culture to a standstill.

If, of course, the food value of the flour is reduced to zero, energy slowly leaves the system through respiration and heat loss (operating through Ξ) until the beetles die, ξ being reduced to zero. This of course is analogous to the fallacy of attempting to rear chickens by feeding them on their own eggs.

**The weight and per cent of nutritive material in an egg are not included as these are probably very constant at constant temperature.*

Direct Examination of Cannibalistic Egg Eating

One may examine the problem from another viewpoint by considering cannibalistic eating directly. From (2, Equation 24, p. 648) the rate at which eggs are consumed by the N_{11} adults is

$$C'_{11.2} = \frac{E_{11} P_{11\cdot 2} r^2_{11.2} N_{11} N_2}{P_{11\cdot 2} A_2 W_2 r^2_{11.2} N_2 + P_{11\cdot 1} A_1 W_1 K_{11} r^2_{11.1} G} \tag{6}$$

from which it is at once seen that cannibalistic eating increases with a decrease in either the palatability of the flour ($P_{11\cdot 1}$) or its nutritive content (A_1). One would not expect this change to occur instantaneously when poor flour is substituted for good flour because the body of the beetle has certain reserves, and some mitigation of impending starvation can be obtained by reduction in the numbers of eggs laid (see below) and these will act as damping influences on any sudden attack on the eggs.

If A_1 be too low, E_{11} must inevitably be reduced, though it cannot fall below or even to Ξ if the culture is to continue to exist. The value of $A_{1(0)}$ will depend then to some extent on how much E_{11} is reduced through reduction in egg laying.

Inasmuch as $\epsilon A_2 W_2$ is relatively large in proportion to $A_{11} W_{11}$, it is probable that some such reduction in E_{11} occurs very soon after the beetles are placed in flour of low nutritive value, and it is suggested that this may explain the more immediate fall in egg numbers observed by Park (1) on placing beetles in conditioned flour, the later cumulative effect being due to increased cannibalistic eating in the endeavor to keep the rate of food intake above Ξ in the face of falling A_1.

Effect of a Decrease in Nutritive Value of the Flour on ξ

From (2, Equation 30, p. 649)

$$\xi = -\frac{b}{a} = \frac{R\epsilon N_{11} P_{11\cdot 1} A_1 W_1 K_{11} r^2_{11.1} G}{E_{11} P_{11\cdot 2} r^2_{11.2} N_{11} - R\epsilon N_{11} P_{11\cdot 2} A_2 W_2 r^2_{11.2}} \tag{7}$$

which may be written in the form

$$\xi = \frac{\alpha A_1 P_{11\cdot 1}}{\beta} \ . \tag{8}$$

Clearly then ξ decreases with a decrease in the palatability or nutritive value of the flour.

Changes Resulting from Denser Packing of the Flour

While it is not contended that the conditioned flour used by Park (1) is appreciably more or less densely packed than normal flour, it is of interest to examine at this point the effect of changes in the degree to which the flour is packed down, on the population.

If the flour be packed more tightly, the volume G being kept constant, it will be seen from Equation (6) that cannibalistic eating will decrease, and thus the number of eggs present at a given time will increase. Moreover, the number of eggs will increase by reason of protection from eating due to the fact that the more densely packed flour is, in effect, more nutritious per unit volume. This effect could also have been shown from Equation (1)

as $\frac{\partial N_2}{\partial W_1} > 0$. Similarly it can be shown that ξ increases with denser packing.

It might be thought that this effect would be due to the fact that as W_1 is increased, μ_{11} the speed of movement of the beetles, is decreased, and so, from (2, Equation 8, p. 644)

$$M'_{11.2} = \frac{N_{11} N_2 \mu_{11} \pi r_{11.2}^2}{G}, \tag{9}$$

the number of eggs encountered per unit time, is less, and so fewer eggs are likely to be eaten. It must be remembered, however, that for small changes in μ_{11}, $M'_{11.2}$ is independent of μ_{11}.

If large changes in μ_{11} occur, the assumption made in (2, p. 645) that "a steady stream of material passing across the field of perception gives an impression proportional to the distance traveled by the stream in unit time" is probably not justified, and $C'_{11.2}$ becomes a function of μ_{11}. Thus the factor of lessened egg-finding may be important in very tightly packed flour.

Conclusions

It is seen that the findings of Park (1) regarding the reduction in numbers of eggs in populations of *T. confusum* made up in flour of low nutritive value are confirmed by mathematical analysis.

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THE CAUSE OF BOLTING IN SWEDE TURNIPS (*BRASSICA NAPUS* VAR. *NAPOBRASSICA* (L.) PETERM.)[1]

BY F. H. PETO[2]

Abstract

The relation of growth temperature to "bolting" (premature seed-stalk development) in swede turnips was investigated by growing plants in a greenhouse at high and low temperatures for different periods of time at various stages of their development. It was found that an initial growth temperature of approximately 53° F. for 30 to 50 days induced incipient bolting 71 days after seeding, as indicated by a rapid increase in internode length accompanied by a retarded root development. Flowering was inhibited to a large extent by growth temperatures above 65° F. in plants that had shown incipient bolting as a result of previous low-temperature treatments. None of the plants grown continuously at high temperatures gave any indication of bolting. It may be concluded, therefore, that high temperatures favor vegetative growth while low temperatures favor sexual reproduction.

Introduction

The swede turnip, being a biennial plant, normally develops during the first season of growth a large fleshy root with a compact cluster of leaves borne on a very short neck or stalk. If it is allowed to continue growth in the second year, it produces a tall, branched top growth bearing the inflorescence and, subsequently, seed.

It occasionally happens that some plants in a crop grown from presumably pure swede turnip seed produce inflorescences and seed the first season of growth. These plants are undesirable on account of their small root size and are commonly known as "bolters". They have, however, more than a superficial resemblance to rape and on this account the grower frequently suspects them to be such and therefore criticizes the seed merchant for selling him impure seed. These complaints were frequently referred to the Seed Branch of the Dominion Department of Agriculture and at their suggestion the problem of determining the cause of this abnormal development was undertaken in the National Research Laboratories.

The appearance of these abnormal plants or bolters in a field of swede turnips may be due to any of the following four conditions: (1) an admixture of rape or swede-like rape; (2) the presence of segregates of natural crosses between any two of swede turnips, rape and swede-like rape; (3) the presence of strains of pure swede turnips with a genetical predisposition towards bolting; (4) environmental conditions which cause a tendency to seed production rather than root production.

There are numerous publications on the cause of bolting in beets, cabbage and celery, and the majority of these indicate that low growing temperatures are primarily responsible. Only one paper was found (12) which referred to

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bolting in swede turnips, and this suggested early sowing in frosty seasons as the main cause. It seemed, therefore, that a study of the effect of temperature on growth habit would provide valuable information, and since greenhouse facilities were available for a controlled experiment this phase of the investigation was undertaken.

Literature Review

Lafferty (12) ascertained the nature of certain "rogues" found among crops of swede turnips in Ireland by selecting representative specimens and inbreeding them. In addition to true yellow-fleshed swede turnips of the green- and purple-top varieties, he identified typical rape and segregates of natural hybrids between swede turnips and rape. Lafferty claimed that these rogues were either the result of an admixture of rape seed with subsequent natural crossing with swede turnips or the result of cross fertilization between adjacent fields of rape and swede turnip. He also recognized that bolters occurred in the swede turnip crop that were not due to the above causes but rather to unusual environmental conditions. He suggested that their occurrence was intimately bound up with, if not entirely due to, unduly early seeding and the incidence of late frosts which tended to check the growth of the young plants. Lafferty claimed that the above two types of bolters should be unmistakably distinguished by certain morphological characteristics. The yellow colored flesh and the buff or "Naples yellow" colored flowers of the swede should readily distinguish the environmental bolters from the natural hybrids which usually exhibit the rape characteristics of "lemon yellow" flower color and white fleshed roots.

A valuable contribution to the knowledge of color inheritance of the various organs of swede turnips was made by Davey (6, 7) and his findings in this regard should be useful in guarding against contamination of swede crops by rape or swede-like rape.

Catcheside (3) studied the chromosomal relationships in the swede and turnip groups of *Brassica*. He found that a close relationship between swedes, swede-like rapes, and bulbless bolters was indicated by their having the same chromosome number ($2n = 36$), and that this close relationship was further emphasized by the conditions at meiosis. All the three groups of plants and their F_1 hybrids usually formed 18 bivalents at meiosis, showed similar degrees of secondary pairing, had approximately the same chiasma frequency at mid-diakinesis, and all formed 100% good pollen. The bulbless bolter which was studied by Catcheside was probably either a swede-like rape or a hybrid similar to those observed by Lafferty (12).

Though very little information is available on the influence of environmental factors on the incidence of bolting in swede turnips, we may form some estimate of their probable effect by a consideration of work done with other crops.

Gassner (10) studied the effect of temperature on the incidence of flowering in *Beta vulgaris* L., *Brassica oleracea* L., *Brassica Rapa* L. (fall turnip),

Brassica napus L. (winter rape), and others. Seed of these plants was sown in October and November in the greenhouse at 20° C. and grown at this high temperature until January 3. The plants were then divided into two series, one placed in the cold house at near the natural winter temperature, the other left in the warm house at about 20° F. In the middle of April all the plants were placed in the open. Only those plants which were in the cold house during the winter flowered during the following summer.

Miller (13) found that a relatively low temperature during the early stages of growth stimulated seed-stalk development in the cabbage. He tried the effect of increasing the length of day five hours by using electric light during the short days of fall and winter but found that this had no effect on seed-stalk development either in the young plants or the mature cabbage. Boswell (1) also found that the earlier cabbage was sown the greater the tendency to go to seed. In a later publication (2) he concluded that a period of exposure to low temperature was requisite for flower formation and that this low temperature was effective in promoting reproduction only after the plant had accumulated a sufficient weight of reserve foods. This accounted for the observation that seed-stalk formation was closely associated with the size of the plant at transplanting time. Heavy applications of nitrogenous fertilizer increased the size of the plant at the time of transplanting and the larger plants were observed to produce seed stalks sooner than the smaller plants. Attempts were made by Boswell to determine whether a correlation existed between the C : N ratio and the tendency of cabbages to shoot to seed. The initial experiments showed an apparent positive correlation. Subsequently an attempt was made to lower the C : N ratio by a heavy application of nitrogenous fertilizer and thereby prevent premature seed-stalk formation. The results showed that the apparent correlation between chemical constitution and the tendency to shoot to seed was a chance parallelism since, when the C : N ratio was reduced, the expected reduction in the number of seeders was not obtained.

Thompson (16, 17, 18) found that young celery plants subjected to relatively low temperature (40° to 50° F.) for two weeks or longer were likely to seed prematurely. However, growing temperatures above 70° F. prevented seeding, regardless of the previous low temperature treatment. He also found that a serious check in growth by freezing or drying tended to delay rather than hasten seed setting. Crowding of the plants had no positive effect on seed-stalk formation.

The recent findings of Chroboczek (4, 5) with reference to seed-stalk formation in beets have perhaps the closest relation to the present investigation of any of those described. His experiments also have an advantage over most of the previous work, in that he studied simultaneously the effect of temperature and light. He found that all the plants grown at 50 to 60° F. went to seed in the first year, while at the medium temperature (60 to 70° F.) only a few plants went to seed under the influence of the long day in the late spring months. At a high growing temperature all the plants developed

vegetatively. He also found that a treatment period of 30 days or longer at 40–50° F. induced flowering if the plants were subsequently grown at 60 to 70° F. under long day conditions. If, however, they were grown at temperatures of 70–80° F. subsequent to treatment, the effects of the previous cold treatment were nullified. Chroboczek also made the interesting discovery that the effect of low temperature treatment on seed development seemed to be localized in the region of the growing point. Premature seeding was induced in plants grown at relatively high temperatures by cooling only the region of the growing point to about 57° F. Another experiment by this author proved the importance of a long light duration to seed-stalk formation. When germination was commenced on January 15, seed-stalk formation had begun in one lot under continuous illumination on March 5, but under conditions of natural daylight, seed-stalk development did not commence until April 23. Munerati (14) was able to produce five generations of beets within one year by supplementing the daylight by light of high intensity during the night. Under these conditions seed stalks formed on seedlings with only two leaves. This author unfortunately fails to give any information about the other environmental factors in his unique experiment.

Garner and Allard (8, 9) studied photoperiodism in a large number of plants and came to the conclusion that a condition of balance exists between reproduction and vegetative activity. Plants were classified as long- or short-day plants according to their reaction under different lengths of day. It was found in one of their experiments (8) that a variety of soy beans required only 23 days to flower when receiving five hours of daylight while 62 days were required when the daily exposure was increased to 15 hr. Another experiment by the same authors (9) showed that reducing the growth temperature from approximately 72° to 55° F. delayed or inhibited flowering in soy beans. They also proved, for the plants they studied, that the duration of daylight rather than intensity was the important factor in exerting a controlling influence on the attainment of the reproductive stage. A reduction in intensity to one third or one fourth of normal sunlight did not modify the plant behavior to any extent. A brief reference was made by Garner and Allard to bolting in beets. They stated that the beet ordinarily is a biennial in the latitude of Washington, but that it completes its life cycle in a single season when grown in Alaska where the summer days are very long.

Very little is known of the relation of heredity to bolting in swede turnips. Differences have been observed, however, in the frequency of bolting between varieties grown under the same environmental conditions. There is an unconscious selection of non-bolters in agricultural practice, since the seed from bolters is never used. Bolters, however, have never been eliminated and the reason for this would appear to be that strains have never been selected consistently under conditions of temperature and light which have been favorable for bolting.

Thompson (18) found wide differences between varieties and strains of celery in respect to bolting, but these genetical differences were frequently

masked by the effects of environmental conditions. Van Heel (19) inbred strains of sugar beets with few and many bolters. From his results from crossing these strains he concluded that the factors for bolting were recessive. Sutton (15) crossed a green non-bolting variety of cabbage with a red bolting variety and obtained a segregation which approximated the Mendelian 9 : 3 : 3 : 1 ratio, green color and bolting being the recessive characters. Sutton, however, realized that seasonal and cultural factors were capable of influencing the frequency of bolting.

Hall (11) published the results of researches carried out at John Innes Horticultural Institution by Professor Bateson who, by inbreeding and selection of sugar beets and mangolds under conditions favorable for bolting, was successful in almost eliminating this characteristic. Hall does not believe that high resistance to bolting is either dominant or recessive, the evidence showing rather that bolting is governed by several factors. The degree of stimulus necessary to cause bolting differs with varieties and this character is inherited. The ordinary seed stock is supposed to consist of a mixed population, the members of which may respond differently to a given environment.

Methods

A greenhouse experiment was designed to test the relation of various growth temperatures to the incidence of bolting. The plants were exposed to eleven different treatments during a period of 50 days from the date of seeding. The mean temperatures in the two greenhouse rooms employed are shown in Fig. 1, the points being calculated from mean bi-hourly temperatures read from thermograph records. For the 50-day period they averaged roughly 53° and 70° F. The post-treatment temperatures of the cold and warm rooms are shown in Fig. 2. Here the temperature differential becomes progressively less, as the onset of warm spring weather made the maintenance of low temperatures impracticable. The average differentials for the treatment and post-treatment periods were thus about 17° and 7° F.

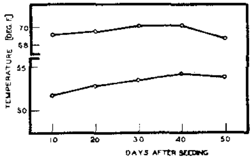

Fig. 1. *Mean temperatures in the cold and warm rooms during the treatment period.*

On the completion of the treatment period, one half of the replicates of each treatment were maintained in the warm room and the other half in the cold room. The designation of post-treatment in the cold or warm room in the diagrams, therefore, refers only to the 100-day maintenance period subsequent to the 50-day treatments, the latter being designated by the numbers 1 to 11, the key to which is given later.

Two varieties of swede turnips were used, namely, Variety A "Hall's Westbury" and Variety B "McDonald's Perfecta". Twenty replicates were placed in each treatment, making 220 plants of each variety on test. The

seeds were sown in 4-in. pots, three seeds per pot, and later thinned to single plants. At the end of the 50-day treatment period all the plants were re-potted into 6-in. pots and left until the completion of the experiment. The location of the replicates on the bench was randomized to compensate for border effect and varying intensities of illumination. A different random distribution had to be calculated for each change of treatment.

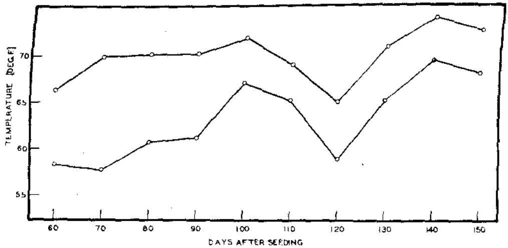

FIG. 2. *Mean temperatures in the cold and warm rooms during the post-treatment period.*

The experiment was commenced on January 21, 1934, and terminated on June 21. Normal daylight was supplemented by artificial light for eight hours from January 21 to April 5. On the latter date the artificial illumination was reduced to five hours and on May 1 it was eliminated. It was necessary to add 5 gm. of chemical fertilizer to each pot on May 21 in order to compensate to some extent for the rapid depletion of fertility due to the crowded condition of the root systems in small pots. This fertilizer consisted of superphosphate 10 parts, nitrate of soda 4 parts, muriate of potash 3 parts.

The date of emergence of the seedlings in each treatment was recorded and the stage of plant development was noted every 10 days during the treatment period by recording the number of leaves per plant. The incipient stages of bolting were indicated by a rapid increase in internode length and a slow increase in root size in certain treatments while the converse was true of the remainder of the treatments. Measurements of internode length and root diameter were taken when this differentiation was first observed. On the completion of the experiment the weights of the roots and tops as well as the internode length were recorded.

The various conditions for the 50-day treatment period are indicated in Table I.

TABLE I

Treatment no.	Description
1	High temperature for 50 days
2	Low temperature for 10 days, high for 40 days
3	Low temperature for 20 days, high for 30 days
4	Low temperature for 30 days, high for 20 days
5	Low temperature for 40 days, high for 10 days
6	Low temperature for 50 days
7*	High temperature for 10 days, low for 10 days, high for 30 days
8*	High temperature for 20 days, low for 10 days, high for 20 days
9*	High temperature for 30 days, low for 10 days, high for 10 days
10*	High temperature for 40 days, low for 10 days
11	High temperature for 50 days, with the exception of 16 hr. at 0.1° C. after 25 days' growth

**The plants in Treatments 7 to 10 were also exposed for 16 hr. at 0.1° C. on the fifth night of the low temperature treatment.*

Observations

In the low-temperature room it required an average of 15 days for Variety A and 17 days for Variety B to reach the stage where 50% of the plants had emerged. In the high-temperature room it required only 6 and 7 days respectively for Varieties A and B to reach this stage.

The growth during the 50-day treatment period was of course greater in the plants which had been grown at the high temperature for the longest period. For example, the plants in Treatment 1 had reached the 8- or 9-leaf stage, while the plants in Treatment 6 had only reached the 6-leaf stage at the end of the treatment period. An exposure of 10 days at the lower temperature had no noticeable effect on plant growth regardless of the stage of growth at which this exposure was made (Treatments 2, 7, 8, 9 and 10). The plants in Treatments 4, 5 and 6 which were placed in the cold room for 30, 40 and 50 days respectively were appreciably smaller than the remainder at the end of the treatment period.

The first evidence of any tendency to bolting was observed 21 days after the completion of the treatment period. The plants of the four most prolonged cold treatments (20, 30, 40 and 50 days) were noticeably different from the remainder in that the internodes had begun to lengthen and there was little or no increase in root diameter. In comparison, the plants grown for longer periods at the higher temperature had commenced to develop normal roots and there was no pronounced elongation of the internodes. At this time measurements of the root diameter of all the plants were taken at the point of greatest enlargement above the surface of the soil. Measurements of the total internode length were also made. It was considered that the internode length divided by the root diameter would give a quotient which would be indicative of the bolting tendencies of the plants so measured. The main advantage of this quotient is that it presents a picture of the relative development of root bulb and stalk regardless of the size of the plant

which, of course, is also influenced by the treatment. The significance of the treatment differences so obtained was tested by the calculation of the standard error.

TABLE II

MEAN OF TOTAL INTERNODE LENGTH DIVIDED BY ROOT DIAMETER
(71 days after seeding)

Treatments	Variety A, post-treatment in warm room	Variety B, post-treatment in warm room	Variety A, post-treatment in cold room	Variety B, post-treatment in cold room
	Mean S.E. of M.	Mean S.E. of M.	Mean S.E. of M.	Mean S.E. of M.
1	1.52 ± 0.15	2.08 ± 0.43	1.45 ± 0.18	1.50 ± 0.21
2	1.82 ± 0.23	1.94 ± 0.25	1.50 ± 0.16	1.42 ± 0.09
3	2.40* ± 0.33	3.15 ± 0.83	2.76* ± 0.48	2.12* ± 0.15
4	4.46* ± 1.00	4.20* ± 0.66	3.04* ± 0.52	2.98* ± 0.36
5	4.30* ± 0.53	4.88* ± 0.85	3.21* ± 0.42	3.15* ± 0.41
6	4.70* ± 0.80	5.62* ± 1.13	3.70* ± 0.46	3.10* ± 0.36
7	2.06* ± 0.20	1.68 ± 0.13	1.62 ± 0.29	1.66 ± 0.19
8	1.94 ± 0.30	1.64 ± 0.15	1.95 ± 0.49	1.33 ± 0.18
9	1.77 ± 0.14	1.46 ± 0.13	1.28 ± 0.18	1.45 ± 0.30
10	1.50 ± 0.18	1.88 ± 0.35	1.80 ± 0.15	1.23 ± 0.12
11	1.87 ± 0.19	1.31 ± 0.13	1.29 ± 0.16	1.16 ± 0.10

**Mean of treatment significantly higher than that of Treatment I.*

According to Table II the quotients calculated for Treatments 3, 4, 5 and 6, which included an initial period of 20, 30, 40 and 50 days respectively in the cold room, were all higher than those for Treatment 1, significantly so with the one exception of Treatment 3, Variety B, post-treatment in warm room.

The only other quotient significantly higher than the corresponding value in Treatment 1 is that for Treatment 7, Variety A, post-treatment in warm room. This is the only instance where 10-day low-temperature treatment induced any tendency to bolt and it will be noticed from later records that this difference was subsequently eliminated.

The high ratio of internode length to root diameter is considered to be definite evidence of incipient bolting. The pictures in Plate I were taken two weeks after the observations were made and they show the differences in type of development between typical plants of the 11 treatments. The lack of root development and the long internodes can be seen in the plants of Treatments 4, 5 and 6.

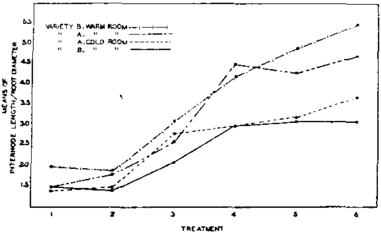

FIG. 3.

Variety B. *Representative plants of treatments 1 to 11, 86 days after seeding.*

Fig. 3 illustrates the differences brought out by statistical analysis of Treatments 1 to 6. The fact that a tendency to bolt can be detected thus early in development does not necessarily indicate that these plants will bolt regardless of subsequent environmental conditions. A high maintenance temperature may check seed-stalk development and initiate storage-root development. Some evidence of this was observed at a later stage of development when plants which had shown a very marked elongation of internode length during earlier development at lower temperatures, began to show a decreased internode length between freshly formed leaves, and additional root development, after being exposed to higher temperatures.

Inflorescences appeared on three plants of Treatment 6, Variety A, and two plants of Treatment 6, Variety B, between 99 and 135 days after seeding, where the plants were subjected to the post-treatment in the cold room. Under conditions where a higher maintenance temperature was employed after treatment only one plant came into flower. This was found in Treatment 6, Variety A, 130 days after seeding. This difference indicates the sensitivity of these plants to comparatively small temperature differences, as there was an average temperature difference of only 5° F. between the low- and high-temperature rooms during the flowering period.

The experiment was terminated on June 21, since it was apparent that no more plants would flower under the high temperatures which existed in both rooms at this time. The effect of the treatments on the plant type nevertheless could be clearly seen at this time as illustrated by the contrast between the plants in B and D, Fig. 4. The plant C has not produced an

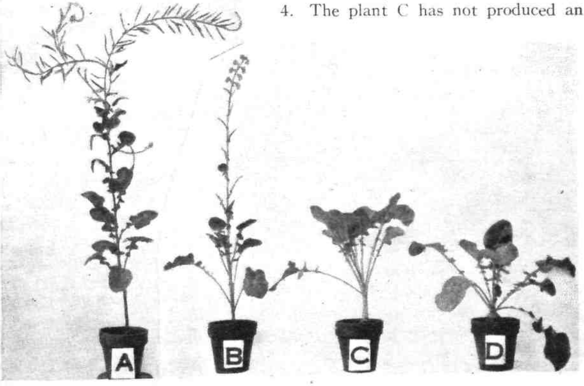

FIG. 4. *A. Variety* B. *150 days after seeding. Treatment 6 followed by growth in cold room. B. Same plant, 113 days after seeding. C. Variety* B, *113 days after seeding; Treatment 6 followed by growth in warm room. D. Variety* B, *113 days after seeding; Treatment 11, followed by growth in warm room.*

inflorescence but it can be definitely classified as a bolting type. Observations were taken at the completion of the experiment on root and top weight, internode length, and the ratio between root weight and internode length.

TABLE III

FINAL MEAN ROOT WEIGHT

(5 months after seeding)

Treatment	Variety A, post-treatment in cold room, gm.	Variety B, post-treatment in cold room, gm.	Variety A, post-treatment in warm room, gm.	Variety B, post-treatment in warm room, gm.
1	75.2	94.2	61.5	62.3
2	71.4	68.9	52.8	84.9
3	60.4	63.1*	47.0	76.0
4	40.9*	78.3	34.4*	44.0
5	25.9*	40.2*	22.0*	37.2
6	34.5*	53.9*	36.4*	42.2
7	61.0	92.3	59.4	84.4
8	61.0	83.1	56.9	81.4
9	61.3	96.0	48.2	79.6
10	113.6	130.2	74.5	117.5
11	68.2	78.7	65.1	102.9
S.E. of difference between two treatment means	±10.5	±14.1	±9.8	±13.8

**Mean root weight significantly lower than that of Treatment 1.*

TABLE IV

RESULTS OF ANALYSIS OF VARIANCE OF ROOT WEIGHT

Variety	Post-treatment room	Variance between treatments	Variance within treatments	S.E. of one treatment mean, gm.	S.E. of difference between two treatment means, gm.
A	Cold	5,189.23	547.11	7.4	10.5
B	Cold	5,817.12	989.83	10.0	14.1
A	Warm	2,322.80	480.96	6.9	9.8
B	Warm	6 446.72	951.66	9.8	13.8

Table III gives the mean root weights of 10 plants for each variety, treatment and post-treatment. The results of an analysis of variance of the 220 individual root weights are shown in Table IV. In spite of the high variation within the treatments it is apparent that the variance between the treatments is significantly higher. The calculation of the standard error of the difference between two treatment means permitted an examination of the mean root weights to determine which of the temperature treatments had significantly depressed or increased the root yield over Treatment 1 (the plants of which were kept in the high-temperature room at a mean temperature of approxi-

mately 70° F. throughout the treatment period). The asterisks in Table III indicate the mean root weights that were significantly lower than that of Treatment 1. For Variety B, post-treatment in warm room, none of the root yields were significantly lower than in Treatment 1, the statistical comparison being influenced by one abnormally small root in Treatment 1. However, the mean of Treatments 4, 5 and 6 is significantly lower than the mean of Treatments 1 and 2, the values being: mean of Treatments 4, 5 and 6=41.1 gm.; mean of Treatments 1 and 2=73.6 gm.; standard error of the mean difference between two and three treatments =8.49 gm. The data in Table III, as well as Figs. 5 and 6, indicate that an initial mean growth temperature of approximately 53° F. for 30, 40 and 50 days (Treatments 4, 5 and 6) has a depressing effect on yield. The top weights, on the other hand, were maintained or even increased under these conditions.

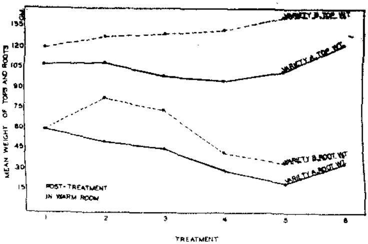

Fig. 5.

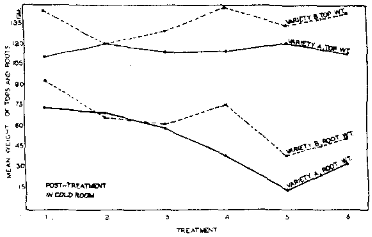

Fig. 6.

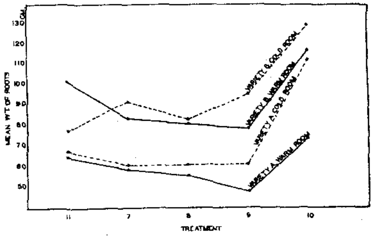

Fig. 7.

In Treatments 7, 8, 9 and 10 the plants were given 10 days in the cold room when 10, 20, 30 and 40 days old respectively. The root weights are shown in Fig. 7 and indicate that a low-temperature treatment after 40 days in the warm room (Treatment 10) stimulates root development, while the same treatment at earlier stages has no significant effect. When

Treatments 7, 8 and 9 were grouped, it was found that the mean of Treatment 10 was significantly higher in all cases as shown in Table V.

TABLE V

Variety	Post-treatment room	Mean root weight of Treatments 7, 8 and 9, gm.	Mean root weight of Treatment 10, gm.	S.E. of difference between mean of one and three treatments, gm.
A	Cold	61.1	113.6	± 8.1
B	Cold	90.3	130.2	±11.0
A	Warm	54.8	74.5	± 7.6
B	Warm	81.8	117.5	±10.7

TABLE VI

FINAL MEAN INTERNODE LENGTH

(5 months after seeding)

Treatments	Variety A, post-treatment in cold room, cm.	Variety B, post-treatment in cold room, cm.	Variety A, post-treatment in warm room, cm.	Variety B, post-treatment in warm room, cm.
1	4.1	5.0	4.9	6.7
2	5.5	4.6	5.5	4.8
3	7.0	5.8	6.1	7.6
4	11.6	9.3	9.0	6.8
5	10.9	9.7	9.8	11.2
6	39.3	35.8	15.4	21.7
7	6.1	8.1	7.3	6.0
8	7.8	6.8	6.9	3.5
9	5.5	7.2	6.9	3.5
10	8.0	5.4	5.4	5.6
11	5.6	4.2	7.0	3.5

It is of interest to note the internode length of the plants in the various treatments as shown in Table VI and Figs. 8 and 9. There appears to be a gradual increase in internode length for the most prolonged low-temperature treatments. In Treatment 6, the plants maintained in the cold room following the treatment period showed a marked increase in the mean length, owing to the number which actually flowered.

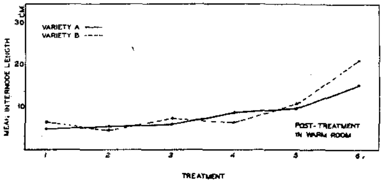

FIG. 8.

It was considered that the degree of differentiation towards bolting could be conveniently expressed by dividing the internode length by the root weight. This quotient was particularly useful since it corrected to some extent for differences in plant size

between treatments. A normal turnip or non-bolter would have a heavy storage root and would have the leaf nodes lying close together, whereas a typical bolter would lack the heavy storage root and would have a tall stalk with the nodes widely spaced. From the data shown in Table VII and also from Figs. 10, 11, 12 and 13 it can be seen that the quotients increase regularly from Treatments 1 to 6 (with two exceptions seen in Figs. 12 and 13). The tendency to bolt is thus correlated with the length of exposure to initial growing temperatures of 50° to 55° F. and increases rapidly when this exposure exceeds 30 days, as is shown by the sharp upward movement of the curve from Treatments 4 to 6.

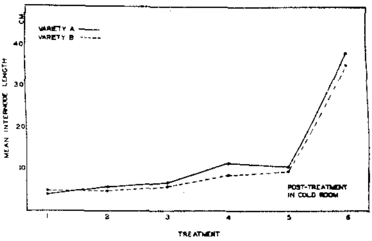

FIG. 9.

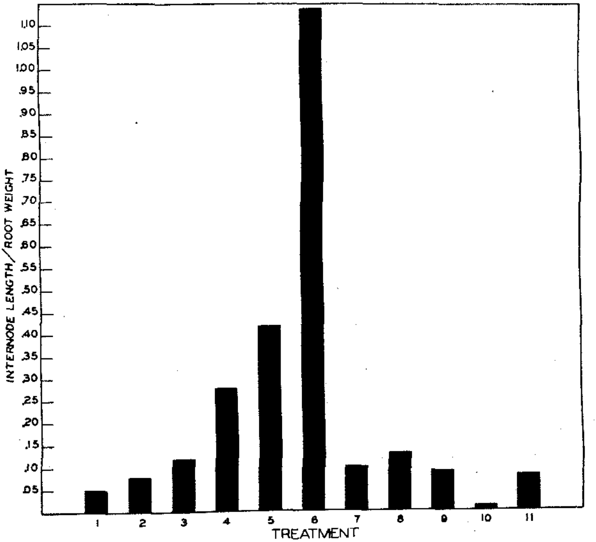

FIG. 10. *Variety A, post-treatment in cold room, mean internode length divided by mean root weight.*

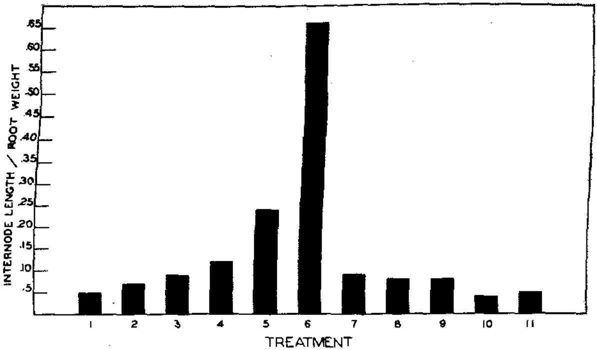

FIG. 11. *Variety B, post-treatment in cold room, mean internode length divided by mean root weight.*

TABLE VII

QUOTIENT OBTAINED BY DIVIDING FINAL MEAN INTERNODE LENGTH BY MEAN ROOT WEIGHT

(5 months after seeding)

Treatment	Variety A, post-treatment in cold room	Variety B, post-treatment in cold room	Variety A, post-treatment in warm room	Variety B, post-treatment in warm room
1	0.06	0.05	0.08	0.11
2	0.08	0.07	0.10	0.06
3	0.12	0.09	0.13	0.10
4	0.28	0.12	0.26	0.16
5	0.42	0.24	0.44	0.30
6	1.14	0.66	0.42	0.51
7	0.10	0.09	0.12	0.07
8	0.13	0.08	0.12	0.04
9	0.09	0.08	0.14	0.04
10	0.01	0.04	0.07	0.05
11	0.08	0.05	0.11	0.03

The post-treatment in the cold room produced more actual seeders than the post-treatment in the warm room and from the data illustrated in Figs. 10, 11, 12 and 13 it is seen that the general tendency to bolt is also more marked under the former conditions. Owing to outside weather conditions, the temperature in the "cold" room actually averaged 65.7° F. during the last 50 days of the experiment, and it may be that a lower post-treatment temperature would have resulted in a much higher percentage of true seeders.

It is interesting to note the character of the low-temperature treatments which failed to exhibit any pronounced evidence of bolting on the completion

of the experiment, *viz.*, (a) an initial cold treatment of 10 or 20 days, (b) a 10-day cold treatment after 10, 20, 30 or 40 days' growth which included 16 hr. at 0.1° C., (c) a cold treatment of 16 hr. at 0.1° C. after 25 days' growth.

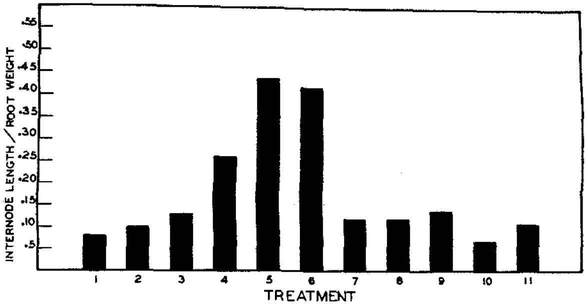

FIG. 12. *Variety A, post-treatment in warm room, mean internode length divided by mean root weight.*

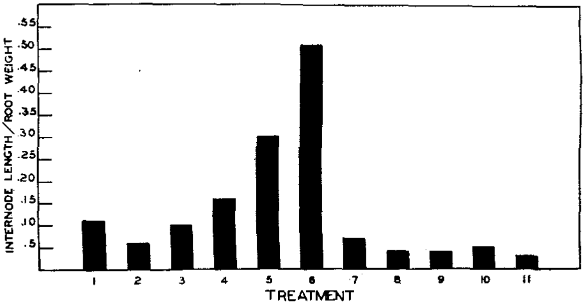

FIG. 13. *Variety B, post-treatment in warm room, mean internode length divided by mean root weight.*

Discussion

The question which suggests itself to the grower is whether growth temperatures which caused bolting in the greenhouse would have a similar effect under field conditions. That is difficult to answer as there are certain obvious differences in the two environments. In the first place, there is probably a higher light intensity in the field which might favor bolting. Whether intensity of light is important or not, it is almost certain that duration is, and this is

continuously changing throughout the season. Second, there are differences in the diurnal temperature variations between the two environments, on which there is no evidence. Differences in moisture and nutrients also may have a minor effect.

Considering temperature only, there is some reason for believing that with varieties with the same genetic susceptibility to bolting as the ones used in this experiment, bolting would occasionally be encountered under field conditions. A rough indication of this is afforded by a consideration of Table VIII which gives the monthly means of bi-hourly readings for May, June, July and August for the years 1918 to 1927 in Montreal. The occurrence of unusually low growing temperatures particularly in May and June may account for the occurrence of bolters in some years. For example, the mean temperature for these months at Montreal was 6.4° F. lower in 1926 than in 1921. A comparison is made of the outside temperatures at Montreal with the cold-room temperatures used in this experiment. The mean temperature during the initial 30-day period in the cold room was approximately 53° F., while the 10-year mean temperature at Montreal for May is 54.9° F. For the succeeding 90 days the mean cold-room temperature was approximately 60° F. and the mean temperature at Montreal was 63.3° F. Since the swede turnip plants grown in the cold room (Treatment 6) all showed a definite tendency to bolt, it might be concluded that the average growth temperature at Montreal would be favorable to bolting if swede turnips were sown on May 1. The usual practice, however, is to sow swede turnips about May 15 and the delay might be sufficient to inhibit bolting in the varieties in question. There would be a difference of 5.5° F. in the mean growth temperature for a three-month period, for seedings of May 1 and June 1 in Montreal. This indicates the possible relation between date of sowing and incidence of bolting.

TABLE VIII

MONTHLY MEANS OF BI-HOURLY TEMPERATURE READINGS RECORDED AT MONTREAL, QUEBEC, ° F.

Year	May	June	July	Aug.	Mean
1918	59.3	61.2	70.0	67.0	64.37
1919	55.5	68.7	70.0	65.4	64.90
1920	57.0	65.5	67.2	70.1	64.95
1921	59.6	66.6	75.4	67.1	67.17
1922	59.0	64.5	69.2	66.2	64.72
1923	53.2	65.0	66.7	64.9	62.45
1924	50.0	64.0	68.6	66.5	62.27
1925	51.0	65.6	66.6	68.8	63.00
1926	52.7	60.7	68.3	66.5	62.05
1927	52.5	61.4	68.3	64.1	61.57
Mean	54.9	64.3	69.0	66.6	

In considering the causes of bolting in turnips, the possibility should not be overlooked that these so-called bolters are in some cases actually rape, swede-like rape, or natural hybrids between either of these and swede turnips. The majority of these plants should be fairly easily distinguished by certain morphological characters not typical of the swede turnip, particularly flesh and flower color as emphasized by Lafferty (12). However, certain segregates of these natural hybrids may be hard to distinguish and a progeny test then

becomes the only positive means of identification. If the so-called bolters cannot be accounted for in this manner and appear to have all the swede turnip characters then one must seek the cause in heredity or environment.

Strains of swede turnips which are particularly susceptible to bolting undoubtedly exist, and a proportion of plants may exhibit this habit even under average growth conditions in the field. If the growing temperatures were unusually low owing either to a cold season or very early seeding, a high percentage of bolters would be expected. On the other hand it is quite probable that most of our turnip varieties have a low susceptibility to bolting and would only show an appreciable number of bolters under conditions particularly favorable to such behavior. These predictions are based on the present greenhouse experiments, the previous observations by Gassner (10) and Lafferty (12), and the experiments on closely related root crops.

Additional greenhouse and field experiments are required to clear up the numerous uncertainties in regard to the causes of bolting, but it appears to be worth while to consider the possible solution of the practical problems from the evidence at hand. The breeder's problem is to select turnips which will have a low susceptibility to bolting. This means selection of biotypes with genetical factors favoring the biennial rather than the annual habit. The chances of accomplishing this would be considerably enhanced by subjecting the breeding material at some stage to environmental factors favoring bolting. The same method should be used to eliminate bolters in recognized varieties. Additional morphological studies of off-type plants and bolters should be made to supply inspectors and growers with information which might enable them to distinguish accurately between off-types that are the result of admixture or hybridization with rape or swede-like rape, and those which are the result of true bolting in pure swede turnips. When an accurate picture of the relative importance of the various environmental factors is available, the grower may be able to make certain adjustments in his cultural practice to reduce the losses from bolting. The most promising solution, however, appears to be in breeding swede turnips which are highly resistant to bolting.

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POLARIMETRIC ESTIMATION OF STARCH[1]

BY CLARENCE YARDLEY HOPKINS[2]

Abstract

For the estimation of starch in wheat and certain wheat products, an adaptation of the polarimetric method of Mannich and Lenz has been developed. It gives satisfactory results with wheat, wheat flour and wheat starch and with mixtures of known composition. Proof is given that the method is accurate in the presence of proteins, sugars and hemicelluloses. It avoids the errors inherent in hydrolytic methods and is believed to be more convenient than Rask's procedure.

Introduction

The exact determination of starch in cereal products is a matter of some difficulty and the methods in common use are not entirely satisfactory with respect to accuracy and ease of manipulation.

The methods of estimating starch may be grouped into four classes: (a) acid hydrolysis, followed by estimation of glucose, (b) enzymic hydrolysis (diastase), followed by estimation of glucose, (c) isolation and weighing of starch, (d) dispersion of starch and estimation by polarimeter.

Acid hydrolysis of cereal products has been shown by Fraps (8) and others to give high results for starch, owing to the production of reducing sugars from pentosans and hemicelluloses. The apparent starch content may be in error by as much as 5% on this account.

Enzymic hydrolysis by diastase is slow and requires correction for a high blank, owing to the presence of sugars in the malt extract used. Herd and Kent-Jones (11) and Rask (17) obtained low results on pure wheat starch by the method of enzymic hydrolysis (diastase). If taka-diastase is employed the blank correction is reduced but the disadvantage of a long digestion period is still present. Furthermore, it has been shown by Herd and Kent-Jones (11) and by Denny (4) that diastatic enzymes will produce reducing sugars from substances other than starch.

The gravimetric methods (c) include those of von Fellenberg (7), Baumert (1), and Rask (17), the last having received the most attention. In Rask's method the starch is dispersed in dilute hydrochloric acid, filtered, precipitated and weighed directly. The results appear to be closer to the true starch content than those of methods (a) or (b). The procedure has been studied and revised by the American Association of Agricultural Chemists (12, 13, 16, 18) but the revised procedure is not entirely satisfactory. It has been adversely criticized by Hall (9) and by Denny (5). A combination of the Rask or von Fellenberg method with estimation by taka-diastase is recommended by Denny (4, 5).

The direct polarimetric estimation of starch was studied by Ewers (6) and by Lintner and Belschner (2). These investigators dispersed the starch in

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dilute hydrochloric acid and used various procedures to obtain a clear solution for polarization. As shown by Hals and Heggenhaugen (10) the acid dissolves certain optically active substances other than starch, which makes the results inaccurate. Furthermore, there is appreciable hydrolysis of starch by the hydrochloric acid during the period of analysis (15).

A new polarimetric method, introduced by Mannich and Lenz (15) in 1920, has received less attention than it deserves. The procedure consists in dispersing the starch in a fairly concentrated calcium chloride solution, filtering and polarizing. The authors give only a few of their analytical results and report an agreement between duplicates within about 10 parts per 1000. The present work was designed to test this method and to determine whether it is applicable to wheat and wheat products.

Mannich and Lenz' Method

The finely ground sample is weighed into a casserole, rubbed up with 10 cc. of water and treated with 60 cc. of calcium chloride solution (two parts crystalline calcium chloride to one part water) and 1 cc. of 0.8% acetic acid. The mixture is boiled gently for 15 min., cooled, filtered, made up to 100 cc. and polarized. In order to correct for the rotation due to soluble proteins and sugars, a separate sample is covered with 100 cc. of calcium chloride solution and stirred at intervals for 30 min. at room temperature. The solution is filtered and polarized. The algebraic difference of the rotations of the two samples gives the rotation due to starch alone. Alternatively, the protein may be removed from the original sample by precipitation with zinc chloride prior to the filtration.

Examination of the Mannich and Lenz Method

The first part of the procedure was found to be very satisfactory and to give concordant results. In attempting to carry out the second part (digestion at room temperature) it was found that some starch was dissolved. Even at 10° C. the filtrate in many cases gave a positive test for starch. Furthermore, it was found that wheat gluten and sucrose do not give the same rotation when dissolved in hot calcium chloride solution that they give when dissolved in cold calcium chloride solution.

Removal of the protein by zinc chloride was also unsatisfactory. The filtrate was cloudy, and there was some evidence of progressive hydrolysis of the starch.

Separation of the starch from protein was attempted by precipitating the starch from the filtrate obtained in the first part of Mannich and Lenz' procedure. Both iodine and alcohol were tried as precipitants and the recovered starch was redissolved in aqueous calcium chloride for polarization. These steps lengthened the procedure considerably, but the most serious difficulty was that precipitation seemed to be incomplete and results were low. The filtrate from the starch-iodide precipitate, although it gave no color with iodine, had a strong positive rotation which could scarcely have been due to anything but starch. One experiment indicated that this unprecipitated

starch amounted to 9% of the starch in the sample. This result is in agreement with the belief that a part of the starch grain does not give a color with iodine (14).

Revision of the Mannich and Lenz Method

It was found that the soluble proteins and sugars are best removed at the beginning by repeated washing with 65% (by weight) alcohol. After this preliminary washing the sample is boiled with calcium chloride solution, using double the amount of acetic acid recommended by Mannich and Lenz. Minor changes have been made in the remainder of the procedure.

The material removed from wheat and wheat flour by the alcohol wash has a negative rotation and in most cases would reduce the apparent starch content by 1 to 1.5% if left in the sample.

The completeness of removal of interfering proteins and of sucrose by this method was proved by the satisfactory analysis of prepared mixtures of wheat gluten and wheat starch and of sucrose and wheat starch. Sucrose has been shown to be the chief sugar present in flour (3).

Analysis of a prepared mixture of starch-free wheat straw and wheat starch showed that the hemicelluloses do not interfere.

The revised method gave excellent checks in duplicate determinations and is fairly rapid, a pair of analyses requiring about 3½ hr. Confirmation of Mannich and Lenz' conclusions was obtained with respect to the following: complete dispersion of starch by boiling for 15 min., absence of hydrolysis of starch during the analysis, and negligible change in specific rotation with temperature changes between 20° and 30° C.

The specific rotation of wheat starch in the solution used is taken as $[\alpha]_D = 200.0$, in accordance with the recommendation of Mannich and Lenz.

It is planned to continue the present work, which deals with wheat starch, to include other cereal starches and potato starch.

The Revised Method

The sample is finely ground (minus 100 mesh if possible) and 2.0 to 2.5 gm. is weighed into a 50 cc. round-bottomed centrifuge tube with lip. It is washed by adding 10 cc. of aqueous alcohol (d_{20}, 0.88) and stirring thoroughly with a glass rod. The rod is placed carefully aside while the mixture is centrifuged and the liquid poured off. The washing is repeated until 60 cc. of wash liquid has been used, stirring each time with the same rod.

The residue is stirred with 10 cc. of water and poured into a 125 cc. Erlenmeyer flask. The transference is completed by washing out with a total of 60 cc. of calcium chloride solution (see below) containing 2 cc. of 0.8% acetic acid. The rod is also transferred to the flask and the mixture is brought to boiling quickly over wire gauze, with frequent stirring. It is boiled briskly

for 15 to 17 min. while precautions are taken to prevent burning and foaming. Particles on the sides of the flask are rubbed down with the rod from time to time.

The solution is cooled quickly in running water and poured into a 100 cc. volumetric flask, rinsing thoroughly with calcium chloride solution (see below) from a wash-bottle with a medium jet. In making up to the mark, it is permissible to destroy the froth by adding one drop of alcohol.

After thoroughly mixing, about 10 cc. is poured on to a fluted filter (Whatman No. 42 or 44), wetting the paper completely. This is allowed to run dry and the filtrate is discarded. Filtration is resumed using a dry receiver, and 40 to 50 cc. is collected.

The liquid is polarized in a 10 cm. tube, taking two sets of 10 readings each. The averages of the two sets should agree within 0.006°.

The starch content is calculated as follows:

$$\text{Starch, \%} = \frac{100 \times A \times 100}{1 \times 200 \times S} = \frac{50 \times A}{S} ,$$

where A is the observed rotation and S the weight of the sample.

The calcium chloride solution is prepared by dissolving two parts of crystalline calcium chloride ($CaCl_2 . 6H_2O$) in one part of water and adjusting to a density of 1.30 at 20° C. This solution contains about 33% of calcium chloride. It is made faintly pink to phenolphthalein by adding $N/10$ sodium hydroxide, and is then ready for use. Anhydrous calcium chloride may be employed but is usually alkaline and requires the addition of acid to bring it to the correct pH.

Experimental

Wheat Starch

The method was applied to a commercial sample of wheat starch (General Chemical Co.) and the non-starch constituents were determined at the same time.

TABLE I

COMPLETE ANALYSIS OF WHEAT STARCH

Non-starch						Starch, %	Total
Moisture, %	Sugars, %	Protein, %	Ash, %	Fibre, %	Fat, %		
10.97, 11.02 Av. 11.00	0.35	0.31	0.21	0.12	0.03	87.80, 87.85 Av. 87.83	99.85

A blank experiment showed that there was no detectable amount of starch in the reagents or the filter paper.

Wheat Flour and Whole Wheat

Wheat flour requires no further grinding. The wheat kernels were ground in a Wiley mill, with special precautions to avoid loss. Care was taken to mix the ground material thoroughly before taking a sample for analysis.

The results of the analyses, along with those for wheat starch, are given in Table II.

TABLE II

STARCH CONTENT OF WHEAT, FLOUR AND STARCH

(13.5% moisture basis)

Material	Description	Starch, %	Mean, %	Difference between duplicates, %
Wheat, hard spring	Marquis, No. 1 Northern	51.75, 52.00	51.88	0.25
Wheat, soft winter	Dawson's Golden Chaff	59.08, 59.02	59.05	0.06
Flour, hard wheat	Marquis	68.01, 68.10	68.06	0.09
Flour, soft wheat	Ben Hur	71.20, 70.97	71.09	0.23
Starch, wheat	Commercial, No. 1	85.33, 85.38	85.36	0.05
	No. 2	85.61, 85.67	85.64	0.06

Analysis of Prepared Mixtures

Mixtures of wheat starch with starch-free substances were prepared and analyzed for starch. The true content of starch in the sample of wheat starch was taken as 87.98%, which figure was obtained by deducting from 100 the sum of the impurities listed in Table I. The starch content of the mixtures is calculated from this figure.

The results, which are given in Table III, show that the method is reliable in the presence of substances which might be expected to interfere.

TABLE III

ANALYSIS OF PREPARED MIXTURES

Material	Starch, %		Error, %
	Calcd.	Found	
Wheat starch	87.98	87.83	0.15
Starch plus wheat gluten	75.82	76.02	.20
Starch plus wheat gluten	79.59	79.73	.14
Starch plus sucrose	79.50	79.39	.11
Starch plus wheat straw	79.73	79.86	.13

The gluten was prepared by washing out a sample of hard wheat flour until the washings no longer contained starch. The wheat straw, which appeared to contain originally about 0.5% starch, was de-starched before use by treatment with malt extract.

Comparison with Rask Method

A comparison of the results obtained by the polarimetric method and the Rask method was made possible through the kindness of V. E. Munsey, who supplied specimens which had been analyzed for starch by the latter procedure.

The comparative figures are shown in Table IV.

TABLE IV

COMPARISON OF POLARIMETRIC AND RASK METHODS

Material	Moisture, %	Starch, % (as received)		Difference, %
		Polarimetric	Rask	
Whole wheat flour	13.36	(56.76, 56.65) 56.71	58.28	1.57
White flour	13.70	(67.69, 67.63) 67.66	68.28	0.62

Comparison with Mannich and Lenz' Method

It was found that omission of the preliminary alcohol wash gave results as much as 1 to 2% too low, owing to the effect of soluble laevorotatory protein.

TABLE V

EFFECT OF SOLUBLE PROTEIN ON RESULT OF STARCH ANALYSIS

(13.5% moisture basis)

Material	Starch, % (mean)		Difference, %
	Without alcohol wash	With alcohol wash (see Table II)	
Wheat, hard spring	(50.41, 50.53) 50.47	51.88	1.41
Wheat, soft winter	(58.87, 58.80) 58.84	59.05	0.21
Flour, hard wheat	(66.30, 66.37) 66.34	68.06	1.72
Flour, soft wheat	(69.68, 69.70) 69.69	71.09	1.40
Starch, wheat No. 1	(85.51, 85.35) 85.40	85.36	0.04

As would be expected, the results on pure wheat starch are the same by the two methods.

Separate samples of these materials were treated with cold calcium chloride solution according to the method of Mannich and Lenz. The results were unsatisfactory owing to the fact that some of the starch was dissolved. Numerous tests were made to find conditions under which starch would not dissolve, but without success. It was found that a temperature of 20° C. or higher is quite unsuitable. Some of the results are given in Table VI.

TABLE VI

RESULT OF TREATING WHEAT AND FLOUR WITH COLD CALCIUM CHLORIDE SOLUTION

Material	Conditions	Iodine test	Rotation
Wheat	30 min. 10° C.	+	−
Wheat	30 min. 20° C.	+	+ (0.012°)
Wheat	30 min. 26° C.	+	+ (0.742°)
Flour	30 min. 10° C.	Trace	−
Flour	30 min. 10° C.	Trace	−
Wheat	After alcohol wash; 30 min. 10° C.	+	+

In further experiments it was found that changing the pH of the calcium chloride solution made a difference in the amount of starch dissolved. In one or two tests the final solution was free of starch and had a negative rotation, but in all other cases a certain amount of starch went into solution.

Moreover, this method of correcting for the soluble proteins assumes that the same amount of protein would dissolve in cold, as in boiling, calcium chloride solution. A simple experiment showed that this is not true. Starch-free wheat gluten (0.350 gm.) gave the following rotation: hot solution, −.066°; cold solution, −.049°. This difference would give rise to an error of about 0.4% in the result for starch.

Similarly, when sucrose is dissolved in cold calcium chloride solution it has not the same rotatory power that it has when dissolved in hot calcium chloride solution. When treated with boiling calcium chloride solution sucrose is partially or wholly inverted. Its rotation after solution in cold aqueous calcium chloride is not far from normal, being affected only by the high salt concentration. Pure sucrose (5.00 gm.) gave the following rotation: hot solution, −1.512°; cold solution, +3.152°.

In several cases, zinc chloride was added to the solution obtained by treating wheat with hot aqueous calcium chloride in order to remove the proteins. There was a precipitate in each case but it was difficult to filter off, and the filtrates were not very clear for polarizing. It was also noted that the zinc chloride was hydrolyzing the starch rather rapidly, as shown by the following figures: rotation after 0 hours, +2.853°; 2 hours, +2.800°; 2½ hours, 2.787°. The use of zinc chloride was discontinued.

Other Experiments

A test was made of the revised method to determine whether hydrolysis of starch occurred. The final solution was polarized at successive intervals, with the following results: rotation after 0 hours, +2.812°; 2½ hours, +2.810°; 7½ hours, +2.801°; 21½ hours, 2.803°. The readings indicate that there is little if auy hydrolysis in the very weakly acid solution.

Wheat starch was dispersed completely in boiling formamide. The solution gelled on cooling, however, and could not be filtered.

A solution of starch in aqueous calcium chloride (rotation, +5.392°) was poured into 2½ volumes of 95% alcohol. The precipitate of starch was filtered off on a Gooch, redissolved in aqueous calcium chloride and brought to the original volume. Its rotation was +5.160°. Evidently the precipitation by alcohol was incomplete.

A solution of starch in aqueous calcium chloride (rotation, +3.506°) was treated with excess of iodine plus potassium iodide and allowed to stand overnight for complete precipitation. The starch iodide was filtered off and the filtrate decolorized with sodium thiosulphate. After it was made up to double the original volume, its rotation was +0.167°, equivalent to +0.334° for the original solution. It gave no color with further additions of iodine.

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CHEMICAL STUDIES ON APPALACHIAN UPLAND PODSOL SOILS

II. ORGANIC MATTER-ACIDITY RELATIONS[1]

BY H. J. ATKINSON[2] AND R. R. MCKIBBIN[3]

Abstract

When leached with distilled water, the raw humus of the A_1 horizon of Quebec virgin podsols gave percolates of gradually increasing pH values over periods of 14 weeks, beginning in two instances at 4.3 and rising to 5.4, and in a third at 3.8, rising to 4.8. When concentrated by evaporation to from one-tenth to one-twentieth of the original volume the percolates retained 83.5 to 99% of their total acidity. The concentrates were fractionated by dialysis through cellophane. From 46 to 91% of the acidity dialyzed. In the dialyzates from five soils, 47.5 to 56.4% of whose acidity dialyzed, total sulphuric acid was found in amounts varying from 30 to 52% of the total acidity. The free (alcohol-soluble) sulphuric acid amounted to 9.5 to 23% of the total acidity of the percolate. Phosphoric acid was present in mere traces in some samples, but in one instance the total phosphoric acid content was sufficient to account for 28% of the total acidity, if it was present in the free state.

Introduction

The characteristic ash-like A_2 horizon of a virgin podsol soil results from severe leaching of the horizon by an acid-reacting solution which percolates from the surface organic matter or "raw humus" A_1 horizon. Practically no information on the composition or nature of this acid solution is available. Glinka (1) stated that "the investigation of the humic material in the podsols shows for horizon A_1 the presence of substances which are known under the names of humic and crenic acids . . . . If the solution reacts acid, the humic acid cannot be extensively removed, for it is soluble only in alkaline solutions. The crenic acid, on the other hand, can be removed to considerable depths by water." Apparently Glinka believed that "crenic acid" is chiefly responsible for the leaching in podsol soils, but with reference to this acid, Neustruev (4) remarked that "no one has proved the presence of crenic acid, and in general its existence and properties are thus far hypothetical." A somewhat similar statement was made by Morgan and Lunt (3) to the effect that "Perhaps we have ceased to believe in hypothetical crenic and apocrenic acids, but we cannot gainsay the action of forest organic matter of strongly acid reaction in the podsolizing process."

The extremely acid nature of the horizon of organic matter accumulation in the podsols is a generally accepted fact. The close relation existing between the organic matter and the acidity of long-cultivated podsols in the Appalachian uplands of Quebec has been pointed out by Shaw and McKibbin (6). They plotted the lime requirement in pounds of calcium oxide per acre, representing the acidity, against the percentage of organic carbon, representing the organic matter, and they found that a direct relation was indicated.

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They showed also that a similar relation exists when the replaceable hydrogen (expressed in milliequivalents) in the base-exchange complex is plotted against the percentage of organic carbon. In undertaking a detailed investigation of the problem of acidity in these soils, it seemed logical to begin the study with the organic matter. In the virgin podsol, it is quite easy to separate the horizon of organic matter accumulation from the neighboring horizons and to obtain a material which is made up almost entirely of the annual deposits of leaves, needles, twigs and other forest litter. This is in a semi-decomposed fibrous condition and has an extremely acid reaction. In the case of every sample subjected to investigation in this work, care was taken to ascertain that a well developed leached layer was present beneath the raw humus layer used. The surface A_0 horizon of undecomposed material was removed, as was any trace of the A_2 leached horizon. The procedure followed in this investigation was worked out step by step, as no satisfactory method was available at the start. Each successive step was carried out in such a way that as little change as possible would be brought about in the original constituents.

Experimental and Discussion

Treatment of Samples

The organic matter of the A_1 horizon, or "raw humus," was first passed through a screen ($\frac{1}{4}$ in. mesh), and roots and other undecomposed material were removed as completely as possible. The screened material was then put into glass percolators, the bottom openings of which were covered with fine gravel which had been previously digested with hydrochloric acid and sodium hydroxide and then carefully washed with distilled water. The gravel served as a support for the soil and did not interfere with the percolation of liquid. Distilled water was added at the surface, and the liquid that came out at the bottom was collected for examination. This method is similar to that employed by Parker (5) in his investigation of the soil solution of cultivated soils. The liquid obtained in this way from the "raw humus" of virgin podsol soils should correspond closely to the material active in bringing about podsolization. In the field, rain water passes through the organic matter layer and carries with it those substances which act on the strong bases and sesquioxides of the underlying horizons, dissolving and leaching them to lower levels, leaving the bleached podsol horizon. The addition of distilled water in the laboratory is analogous to the addition of rain water in the field. Free drainage is permitted in the percolator because the sample is supported only on loose fine gravel. Free drainage can also take place in the field. In the percolator, the raw humus is saturated with water before any liquid comes out at the bottom. Such conditions also exist in the field, especially in the spring after the winter snows have melted, and perhaps also at intervals throughout the summer and autumn after heavy rainstorms.

Distilled-water Percolate

The distilled-water percolate obtained as outlined above is a clear, dilute, somewhat colored, strongly acid solution. Its acid nature is easily shown by

determining the hydrogen ion concentration. Since the solution is colored, this cannot be done colorimetrically. In this work, it has been done electrometrically, using a Leeds and Northrup type K potentiometer, a saturated potassium chloride-calomel half-cell and bubbling hydrogen electrode. Successive percolates taken at intervals of several days over a period of some weeks continued to be strongly acid.

In Table I are presented data obtained with percolates from three different soils. The percolates were taken at intervals of two weeks for a period of 14

TABLE I

DISTILLED-WATER PERCOLATES TAKEN AT INTERVALS OF TWO WEEKS FROM 500 GM. OF RAW HUMUS (WEIGHED AIR-DRY)

	No. of weeks after first addition of distilled water						
	2	4	6	8	10	12	14
Soil No. 1							
pH	4.26	–	4.85	5.21	5.29	5.39	5.36
Total solids							
On evaporation, %	0.31	0.27	–	0.16	0.14	0.15	0.13
On ignition to 650° C., %	0.022	0.029	0.019	0.017	0.014	0.017	0.010
Soil No. 2							
pH	4.32	4.46	4.90	–	5.14	5.63	5.35
Total solids							
On evaporation, %	0.20	0.21	–	0.15	0.13	0.13	0.11
On ignition to 650° C., %	0.017	0.028	0.018	0.022	0.016	0.014	0.010
Soil No. 3							
pH	3.77	3.96	4.29	4.52	4.57	4.75	4.82
Total solids							
On evaporation, %	0.23	0.16	–	0.14	0.14	0.15	0.16
On ignition to 650° C., %	0.027	0.022	0.019	0.020	0.010	0.014	0.015

weeks. There was 500 gm. of air-dried soil in each percolator, and 500-cc. percolates were collected. The origins of the soils are as follows:—

Soil No. 1—Brome County, vegetative cover—mixed, birch predominating.

Soil No. 2—Compton County, vegetative cover—hard maples.

Soil No. 3—Sherbrooke County, vegetative cover—evergreen, chiefly pine.

The results show that, even after a period of 14 weeks, the percolate continued to be extremely acid, although there was a gradual falling off in the intensity of the acidity as indicated by a gradually increasing pH value. The contention that the organic matter is a potential source of acidity in the podsol soils is strengthened by these results.

The figures in the table also give an indication of a gradual decrease in the content of total solids of successive percolates, both on evaporation and on ignition to 650° C. The greater part (about 90%) of the total solids in these percolates was organic in nature and was driven off on heating to 650° C.

Concentration of Percolates

The dilute nature of the percolates made it necessary to concentrate the solutions to obtain convenient amounts with which to work. It was a very easy matter to obtain a large volume of percolate from a given soil by setting up a battery of percolators and displacing the solution from each. The percolates were evaporated in a distilling flask under reduced pressure at temperatures below 30° C. Tests showed that the liquid distilling off was neutral in reaction and that the acidity became concentrated in the liquid remaining undistilled. This method of concentration has a number of disadvantages, especially when large volumes of liquid are to be concentrated. Further investigation showed that it is not necessary to carry out concentration at such low temperatures. When the percolates were allowed to evaporate in porcelain basins on a steam bath until reduced to from one-tenth to one-twentieth of the original volume, a very high percentage of the acidity was recovered in the concentrate. This was shown by titrating aliquot samples of the percolate both before and after concentration. The hydrogen ion concentration of the sample was determined electrometrically and standard $N/10$ sodium hydroxide solution was added gradually, with stirring, from a microburette until the reading on the potentiometer corresponded to that for pH 7. From the titration values so obtained, the amount of sodium hydroxide necessary to neutralize the whole of the acidity present both before and after concentration was determined, and the percentage recovery of acidity calculated. The results obtained with a number of percolates from different samples of raw humus are presented in Table II. They indicate the results that may be expected from this method of concentration.

TABLE II

RELATION BETWEEN THE ACIDITY OF ORIGINAL PERCOLATES AND THAT OF THEIR CONCENTRATES

Soil No.	Volume of distilled water percolate, cc.	Volume of concentrate after evaporation on steam bath, cc.	Titration values, cc. 0.1 N NaOH		% of original acidity in concentrate
			Original percolate	Concentrate	
4	5,850	600	68.0	64.2	94.4
5	17,900	940	683.5	587.4	86.0
6	15,690	780	147.4	123.0	83.5
7	10,000	660	174.2	160.6	92.2
8	8,000	875	271.0	268.7	99.1
9	6,000	650	129.0	121.7	94.4

The origin of each soil is as follows:—

Soil 4—Compton Co., near Eaton Corners; vegetative cover—evergreen.

Soil 5—Jacques Cartier Co., near Ste. Anne de Bellevue; vegetative cover —evergreen.

Soil 6—Compton Co., near Sawyerville, on Scott farm; vegetative cover—evergreen.

Soil 7—Compton Co., near Sawyerville, on Bulwer road; vegetative cover—evergreen.

Soil 8—From same location as Soil 4, but sample taken one year later.

Soil 9—Compton Co., near Sawyerville, on Taylor farm; vegetative cover—evergreen.

The solutions resulting from the concentration of the percolates were very dark brown, colloidal and opaque. Flocculent precipitates of the colloidal materials could be obtained by the addition of solutions of heavy metal salts, such as lead nitrate, copper sulphate and silver nitrate. A flocculent precipitate was also obtained when the concentrated solution was poured into a large excess of 95% ethyl alcohol.

Dialysis of Percolates

The next step in the examination of the concentrated percolate was dialysis. The purpose of this step was to separate the acids in true solution from those in colloidal solution or associated with colloidal particles. For this purpose cellophane was used and found to be a satisfactory dialyzing membrane. A sheet about 20 in. square can be formed into a sac which will easily contain 600 to 700 cc. This was done by folding the cellophane over the bottom of a large beaker, passing a cord around it, removing the beaker and tightening the cord. The sac was suspended in the centre of a large crystallizing basin or inverted bell jar, and the material to be dialyzed was introduced into it by means of a funnel. The sac was then surrounded with distilled water.

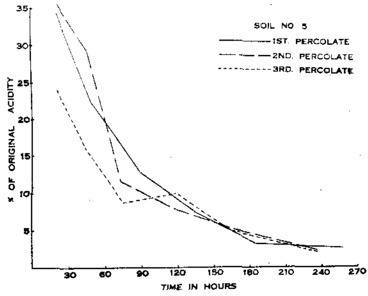

FIG. 1. *Decrease in dialyzable acidity during dialysis for the three percolates from Soil No. 5.*

The water in the dish was changed about once a day. Successive dialyzates over a period of several days showed a gradually decreasing concentration of acidity. Here, as in the previous work, the acidity was determined by electrometric titration of aliquot samples. The dialyzates were colored, owing to the fact that some

organic matter dialyzed, thus precluding the possibility of using colorimetric titration methods. After a period of seven or eight days, the acidity of the dialyzates usually fell off to a very small amount, about 2% or 3% of the original acidity. There was, however, still remaining within the sac a large portion of the acidity which could also be determined by electrometric titration. Thus it was possible to separate the acidity of each percolate into a dialyzable and a non-dialyzable fraction. Furthermore, when the total acidity of the successive dialyzates was added to that of the non-dialyzable fraction, a recovery of usually about 90% of the total acidity was obtained, which may be within the limits of accuracy of the method. Actual figures obtained with two soil samples are given in Tables III and IV, and the results are shown graphically in Figs. 1 and 2.

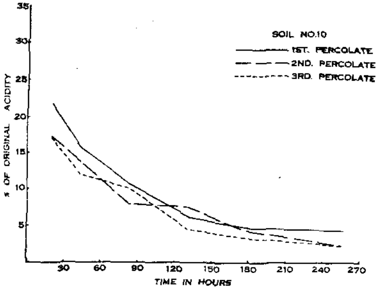

FIG. 2. *Decrease in dialyzable acidity during dialysis for the three percolates from Soil No. 10.*

TABLE III

DATA SHOWING DECREASE OF ACIDITY IN SUCCESSIVE DIALYZATES, SOIL NO. 5. THREE PERCOLATES TAKEN AT INTERVALS OF THREE DAYS

Dialyzate No.	First percolate		Second percolate		Third percolate	
	Time of dialysis, hr.	% of original acidity	Time of dialysis, hr.	% of original acidity	Time of dialysis, hr.	% of original acidity
1	21	34.1	21	35.3	20	24.1
2	28	22.1	24	29.4	24	15.9
3	39	12.9	27	11.6	31	8.8
4	48	7.4	46	7.8	44	10.0
5	49	3.1	48	5.0	46	4.8
6	74	2.5	71	2.1	72	2.0
Total dialyzed acidity		82.1		91.2		65.6
Acidity remaining within sac		12.1		8.7		22.6
Total acidity accounted for		94.2		99.9		88.2

TABLE IV

DATA SHOWING DECREASE OF ACIDITY IN SUCCESSIVE DIALYZATES, SOIL NO. 10.* THREE PERCOLATES TAKEN AT INTERVALS OF THREE DAYS

Dialyzate No.	First percolate		Second percolate		Third percolate	
	Time of dialysis, hr.	% of original acidity	Time of dialysis, hr.	% of original acidity	Time of dialysis, hr.	% of original acidity
1	20	21.8	20	17.4	20	17.1
2	24	15.7	24	13.8	24	12.0
3	39	10.7	39	8.0	39	10.1
4	49	6.1	48	7.5	49	4.5
5	48	4.7	48	4.3	47	3.3
6	77	4.3	78	2.3	78	2.2
Total dialyzed acidity		63.3		53.3		49.2
Acidity remaining within sac		27.1		35.1		37.8
Total acidity accounted for		90.4		88.4		87.0

*Soil No. 10—Compton Co., near Sawyerville, on Halliday farm; vegetative cover—largely evergreen.

Sulphuric and Phosphoric Acids in Dialyzates

Having obtained a definite separation of the acidity, the next step was an investigation of the chemical constitution of the fractions. Little definite information has been obtained on the structure of the non-dialyzable portion. Oxidation with dilute alkaline potassium permanganate solution, even at temperatures below 20° C., yielded only oxalic acid. Other oxidizing agents destroyed its colloidal nature, but so far no substances have been identified which would give any indication of the original structure.

In the dialyzable fraction, the presence of sulphate and phosphate ions has been shown. This indicates that sulphuric acid and phosphoric acid or their acid salts may be responsible for a large part of the dialyzable acidity. Furthermore, the presence of free sulphuric acid has been shown. During recent years, there has been a number of references in the literature to the importance of sulphuric acid in the weathering of rocks. Organic materials are regarded as the source of much of the sulphuric acid present in soils, it being formed as the result of complete oxidation of the sulphur-containing protein substances of plants. One of the most recent papers on this subject is by Heimath (2), who gives a good review of the literature and attributes to Blanck an important part in the investigation of the role of sulphuric acid in soils. Heimath studied the acid organic matter of a number of soils, using a method of electro-ultrafiltration to separate the ions, and showed that the sulphate ion was present in large amounts. He concludes that the free acid must be present to account for the very low pH values obtained.

The distilled-water percolates of a number of soils examined in this work had pH values ranging from 5.5 to 2.9. The most acidic of these, after concentration, showed a value of pH 2.5 for the first dialyzate. Such low

pH values can be caused only by some strong, highly dissociated acid. Hydrochloric acid and nitric acid are never present in sufficient amounts to influence the hydrogen ion concentration to this extent, according to the writers' analyses. There remains the possibility that sulphuric acid and phosphoric acid are responsible. In this investigation, the successive dialyzates of a number of samples have been analyzed for sulphate and phosphate content. The method has been to take the complete dialyzate or a definite fraction of it to dryness in the presence of magnesium nitrate solution as a flux, ignite to about 550° C. to remove all volatile material, take up in concentrated hydrochloric acid, precipitate sulphate as barium sulphate and phosphate as ammonium phosphomolybdate. A blank determination was run on all the reagents used, and the value of the blank was deducted. Sulphate was found in all samples examined, but the presence of phosphate was not so general. It has been found in considerable amount in most cases, but in some it was present in very small quantities only. In Table V are presented the

TABLE V

SULPHATE AND PHOSPHATE CONTENTS OF DIALYZATES, EXPRESSED IN TERMS OF THE ACIDITY OF THE SAMPLES

Soil No.	Percolate No.	% dialyzable acidity accounted for as H_2SO_4	% dialyzable acidity accounted for as H_3PO_4	% total acidity that is dialyzable	% total acidity accounted for as H_2SO_4	% total acidity accounted for as H_3PO_4
5	1st	67.5	1.0	87.1	58.8	0.9
	2nd	23.1	0.4	91.3	21.1	0.3
	3rd	21.1	1.3	74.4	15.7	1.0
6	6th	61.3	17.3	54.6	33.5	9.5
	7th	60.0	20.4	46.4	27.9	9.5
10	1st	90.1	7.2	70.0	63.0	5.0
	2nd	26.9	8.5	60.3	16.2	5.1
	3rd	24.5	15.0	56.6	13.9	8.5
11	1st	140.5	10.5	75.7	106.4	7.9
	2nd	49.9	46.2	75.2	37.5	34.7
12	2nd	79.0	Trace	62.6	49.5	—
	3rd	128.4	Trace	52.2	67.0	—

results obtained for sulphate and phosphate content of the dialyzates of a number of percolates from the "raw humus" horizon of typical virgin podsols. The origin of some of these soils has been given above. Of the others, Soil No. 11 is from Brome Co., two miles from Knowlton, cover—mixed evergreen and deciduous; Soil No. 12 is from Shefford Co., near Stukely, cover—largely evergreen with some deciduous. The total sulphate content has been calculated as sulphuric acid and this, expressed as percentage of the dialyzable acidity, is given in the third column. Similarly, the total phosphate

content, calculated as phosphoric acid, is expressed as percentage of the dialyzable acidity in the fourth column. The fifth column shows, for each sample, the percentage of the total acidity that is dialyzable. From this and the results in the third column, it is possible to calculate the percentage of the total acidity that may be accounted for as sulphuric acid when the total sulphate content is calculated as sulphuric acid. The result is shown in the sixth column. Similarly, from the results in the fourth and fifth columns, it is possible to calculate the percentage of the total acidity that can be accounted for as phosphoric acid, when the total phosphate content is calculated as phosphoric acid. The results so obtained are given in the seventh column.

From the results presented it is seen that the sulphate and phosphate contents of these soils are sufficient to account for a very large portion of the water-soluble acidity, if the acids are present in the free state. One very significant point is that these acids arise entirely in the horizon of organic matter accumulation. They are probably not present entirely in the free state, but partly as neutral or acid salts, owing to the effect of cations of the mineralized part of the organic matter. This conclusion is indicated by the results from Soil No. 11, first percolate, where more than sufficient sulphate is present to account for the entire acidity if it were present entirely as free sulphuric acid. Nevertheless, a considerable amount of these acids must be present in the free state, in order to account for the low pH values of the percolates.

Free Sulphuric Acid

In order to determine what proportion of the sulphate content was present as free sulphuric acid, the solution was concentrated to a small volume (about 50 cc.) and poured into a large volume (about 450 cc.) of 95% ethyl alcohol. Practically all the sulphates were thus precipitated, leaving the free sulphuric acid in solution. Raw humus samples from five virgin podsols, well distributed over the Appalachian upland region of Quebec province, were examined in this way for free sulphuric acid, and these five are the only ones thus far studied in this connection. Three percolates from each soil were taken at intervals of three days, concentrated and dialyzed for 60 hr. The water was changed at 16, 20 and 24 hr. Thus dialysis was not continued until all the dialyzable acidity was removed. Previous work showed, however, that by far the greater part of the acidity is removed in the first three dialyzates, and it is believed that the results obtained in this manner give a good indication of the amount of free sulphuric acid present. Each dialyzate was divided into two equal portions. One was analyzed for total sulphate and the other for free sulphuric acid according to the method outlined above. Total phosphate content was also determined in the fraction that was analyzed for total sulphate.

The results obtained are presented in Table VI and are expressed as percentage of dialyzable and total acidity as was done in Table V. Here, however, the results obtained for the three dialyzates from each of the three per-

TABLE VI

PHOSPHATE AND TOTAL AND ALCOHOL-SOLUBLE SULPHATE CONTENTS OF DIALYZATES, EXPRESSED IN TERMS OF THE ACIDITY OF THE SAMPLES

Soil No.	% dialyzable acidity accounted for as H_2SO_4 from total sulphate	% dialyzable acidity accounted for as H_3PO_4	% total acidity that is dialyzable	% total acidity accounted for as H_2SO_4 from total sulphate	% total acidity accounted for as H_3PO_4	% dialyzable acidity accounted for as H_2SO_4 from alc.-sol. sulphate	% total acidity accounted for as H_2SO_4 from alc.-sol. sulphate
7	69.8	Trace	47.5	33.1	—	20.1	9.5
8	55.3	Trace	55.1	30.5	—	26.4	14.6
9	92.8	Trace	56.4	52.4	—	40.6	22.9
13	78.8	5.7	53.8	42.4	3.1	19.7	10.6
14	57.4	54.2	52.1	29.9	28.2	32.6	17.0

colates of each soil have been added together in order to get one set of figures for each soil examined. The locations of soils Nos. 7, 8 and 9 have been given previously. Soil No. 13 was from Kamouraska Co., about five miles south of St. Onésime. The cover was largely evergreen but contained some deciduous trees. Soil No. 14 was from Montmagny Co., one mile west of St. Paul. The cover was about the same as for soil No. 13. Columns 2 to 6 of Table VI correspond to Columns 3 to 7 of Table V. In the seventh column of Table VI, the sulphate content of the fraction not precipitated on being poured into excess of alcohol has been calculated as sulphuric acid and expressed as percentage of dialyzable acidity. This has been converted into percentage of total acidity of the samples in the eighth column.

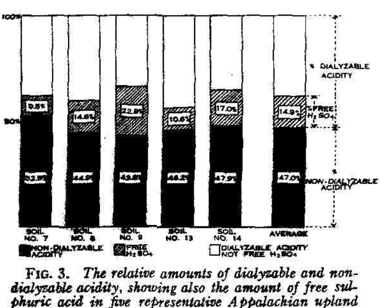

FIG. 3. *The relative amounts of dialyzable and non-dialyzable acidity, showing also the amount of free sulphuric acid in five representative Appalachian upland podsol soils of Quebec.*

In Fig. 3, the results for each of these five soils, together with the average results, are expressed graphically so as to show the relative amounts of acidity present as non-dialyzable acidity (in solid black) and as dialyzable acidity. The dialyzable fraction is subdivided to give the amount of free sulphuric acid determined (shaded). The remainder of the readily dialyzable fraction is represented by the unshaded part. This particular fraction of the acidity is now being studied in this laboratory.

As in Table V, the results here show the presence of phosphoric acid or phosphates in considerable amount in some cases, but only in very small

amounts in others. Sulphuric acid was, however, present in appreciable quantities in all cases, and a large fraction of it existed in the free state. In the five soils examined, sufficient free sulphuric acid was present to account for from 9.5% to 22.9% of the total acidity of the distilled-water percolates. Incidentally, it may be mentioned that the cultivated soil within 100 yards of the place where soil No. 9 (which showed the highest percentage of free sulphuric acid (22.9%)) was obtained, is one of the poorest soils in this region.

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FLOW FORMS IN A CHANNEL OF SMALL EXPONENTIAL DIVERGENCE[1]

By G. N. Patterson[2]

Abstract

The motion of air through a channel of small exponential divergence has been investigated experimentally. A flow form derived by Blasius from theoretical considerations has been shown to exist in the range $25 \leqslant R \leqslant 36$ for the Reynolds number. The dependence of the general flow form on the initial velocity distribution where the divergence begins has been studied. It has been found that when this initial velocity distribution is parabolic, indicating a laminar motion in the throat of the channel, the flow form is symmetrical. Further investigations have shown that when the initial velocity distribution indicates that the motion near the walls in the throat of the channel lies in the transitional region between a laminar and a turbulent flow, then the flow form is unsymmetrical. Empirical equations have been obtained which give (1) the initial velocity distribution in the transitional region at $R=75.1$, and (2) the motion near the walls where the divergence begins for Reynolds numbers lying in the range $0 \leqslant R \leqslant 75.1$.

Introduction

The problem of the flow of a viscous incompressible fluid between two walls which diverge symmetrically according to an exponential relation has been treated theoretically by Blasius (1). The flow was regarded as a steady two-dimensional motion and the walls were considered to diverge very gradually in a manner given by the equation $z=a+be^{\epsilon x}$, where ϵ is small. In order to complete the necessary boundary conditions, some form of initial velocity distribution at $x=0$ was required.

Blasius assumed a parabolic distribution and showed that the divergence of the channel developed a reversed pressure gradient which opposed the motion of the fluid, causing it to break away symmetrically from both walls of the channel. Equations of velocity distribution were obtained which showed the existence of a reversed flow in the neighborhood of the walls. The work of Blasius on this problem also indicated that the flow form between the diverging walls depended upon the initial velocity distribution at $x=0$ in the throat of the channel.

The present experimental work was undertaken (1) to verify the existence of the Blasius flow form and to determine the range of the Reynolds number for which it will be found, and (2) to study the relation between subsequent changes of the flow form and the corresponding variation of the initial velocity distribution at $x=0$.

Experimental Arrangement

Air was chosen as the medium to be investigated. A modification of the photographic method of Nisi and Porter (3) was adopted. A steady motion through the channel was maintained by a large tank from which water

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[2] *This work was carried out while the author held a Research Fellowship in the Graduate School of the University of Toronto.*

siphoned at a constant rate. The flow was made visible by magnesium oxide particles. The region under investigation was illuminated by a flat sheet of light in the horizontal plane and the motions of the particle indicators were recorded on the film of a camera. From the film the length of the particle tracks could be determined, and, using the measured time of exposure, the velocity distribution could then be found.

The method of obtaining a steady flow is illustrated in Fig. 1. The air and magnesium oxide particles are mixed in the box, A, and then allowed

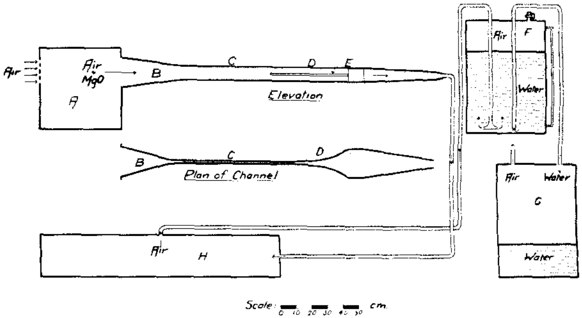


FIG. 1. *Diagram of the general arrangement showing the method of obtaining a steady flow, and the dimensions of the channel.*

to settle so that air currents and large particles of the powder could be eliminated. Water in the tank, F, is permitted to siphon into the tank, G, at a constant rate. The air bubbles into the tank, F, through an inlet which is placed at the same level as the outlet for the water. The velocity can be varied by opening or closing a rubber section of the siphoning tube with a clamp. The mixture of air and particles then passes through the reducing section, B, designed to stabilize the flow, into the narrow channel, C. Through a cellophane window, E, in the divergent section, D, a flat sheet of light is passed which illuminates the central horizontal plane of the channel. The particles are then clearly visible and can be photographed through a glass window fitted on top of the divergent section, D. The mixture continues through a second reducing section into a long pipe and thence into the large reservoir, H.

The end of the pipe through which the air issues into the tank, F, is fitted with four small jets which allow the air to escape into the water in small bubbles which form so rapidly that any tendency of the water to cover the mouth of the jet after the formation of each bubble is checked. This arrangement and the large tank, H, with its long connecting pipes, serve to eliminate

any pressure oscillations due to the formation of bubbles in the tank, *F*. It will be seen that the experiment is arranged so that the powder does not enter the room. Compressed air is used to force the water from the tank, *G*, back into the tank, *F*. The channel is made of wood with the exception of the vertical walls of the divergent section which are made of brass smoothly fitted into place. Over the inside surface of the brass is stretched a thin sheet of cellophane.

It was found that the best photographs of particle tracks were obtained when magnesium oxide powder was used, owing to its lightness and its high reflecting power. If the particles were not too large they remained suspended in still air and the rate at which they settled out was negligible compared with the velocities used in the channel. Fig. 2 shows the method of obtaining a good mixture of air and particles. Compressed air enters the tube, *A*, and mixes with the powder in the container, *B*. The mixture then passes through the tube, *C*, into the chamber, *D*, where it encounters a series of baffles, *E*, which complete the mixing process. It then enters the box, *A*, shown in Fig. 1.

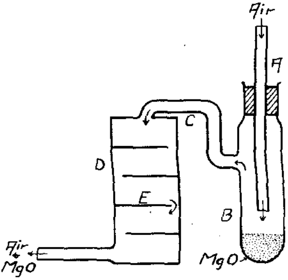


FIG. 2. *Apparatus for mixing air and magnesium oxide powder.*

In the optical system the source of light is an electric arc capable of carrying 30 to 60 amp. The light is collected by a large condenser and transmitted through a water cell. The rays then converge on a narrow slit. The image of the slit is projected through the cellophane windows (*E*, Fig. 1) of the channel by a projection lens. This lens is achromatic and possesses an aperture of 2 in. and a focal length of 2 in. The reflected light from the particles thus illuminated is collected by a photographic lens (2.5 cm. aperture, 5 cm. focal length), and is then projected on the high speed superpanchromatic film of a camera. The camera is placed above the illuminated plane with its axis vertical.

Experimental Technique

Before commencing to take photographs the flow forms corresponding to increases of the mean velocity, $\bar{u}$, from rest up to 22.5 cm. per sec. were observed. Values of $\bar{u}$ at which type flow forms were observed were recorded. Photographs of these type flow forms were taken and then, at corresponding values of $\bar{u}$, photographs were made of particle tracks in the throat of the channel (*i.e.*, in the region of $x = 0$) from which the initial velocity distribution for each flow form was determined. No observations were made when the velocity was higher than the mean velocity of 22.5 cm. per sec., since at greater speeds the particle tracks on the film were not sufficiently clear for accurate measurement.

These observations were made for a narrow channel of very gradual divergence. The straight walls (see Fig. 1) bore a ratio of height to width of 16. This ratio decreased as the channel diverged and it was limited by the short distance from the camera lens to the central horizontal plane of the channel which was necessary for obtaining clear photographs of the particles. The walls of the channel were curved according to the relation

$$\pm z = 0.25e^{0.1x} \quad , \tag{1}$$

where x is the distance along the axis of the channel and $\pm z$ are the distances from the axis to the respective walls. The divergence began at $x=0$, and for $x<0$ the walls were straight and parallel, being separated by a distance of 0.5 cm.

The measurements of velocity distribution required the accurate determination of the time of exposure of the camera and the length of the particle tracks on the photographic film. Shutter speed calibrations were made accurately to 0.0001 sec. by a stroboscopic method immediately after the experiments were complete. A traveling microscope mounted on a cast iron base was used for the measurement of the particle tracks (to 0.001 cm.). The negative was placed between two thin pieces of glass and fitted securely into a holder which was firmly bolted to the cast iron base. The microscope was then accurately aligned. Since the level of the base was adjustable the alignment could be carried out with a level bubble. The determination of the position of each wall on the photographs was aided by observing the position of small particles adhering to the wall. Particles which were out of focus, and therefore not in the plane being measured, were easily picked out owing to the very short depth of focus of the camera lens.

Corrections to the measurements were made whenever they were appreciable. The sources of error were: (1) the fact that the particles could not be regarded as point particles (diameter of the order of 10^{-3} cm.); (2) the coarse grain effects on the negative due to the use of a high speed film; (3) the focal plane shutter of the camera (hardly appreciable even at the highest velocities).

All observations and measurements were confined to the central horizontal plane of the channel. The mean velocity, $\bar{u}$, is that corresponding to this plane and is obtained from the photographs. The photographs of the flow forms are all time exposures of 5 to 10 sec., whereas the photographs of the particle distributions were made at 1/20, 1/30, 1/40, 1/60 sec. All observations were made at a constant room temperature of 23° C.

Results

Photographs of the observed types of flow forms are shown in Figs. 3–6. There are two distinct types: (1) a symmetrical flow (Figs. 3 and 4); (2) an unsymmetrical flow (Figs. 5 and 6). The photographs represent a length of about 25 cm. Since the direction of flow is from left to right it will be seen that reversed flow regions exist which characterize each flow form. These

regions are easily distinguished from the main flow since the density of the particle distribution in the return flow areas is much less than that of the main flow.

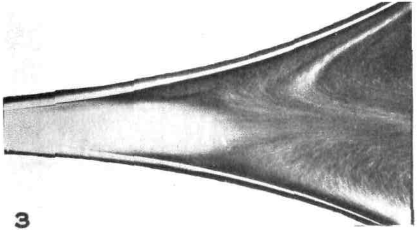
At a mean velocity of 5.6 cm. per sec. the flow form is three-dimensional and symmetrical (Fig. 3). After passing the point where the divergence begins ($x=0$) the main flow develops a downward component of velocity and disappears from the central horizontal plane under observation. This is the white area issuing from the throat of the channel and terminating near the centre of the photograph. In the wide section of the channel two reversed flow regions exist. As the particles enter the field of view they are seen to be moving along the walls toward the narrowest part of the channel. Upon nearing the region where the main flow disappears the particles move away from the walls toward the centre of the channel. This change in position with respect to the walls is accompanied by a rapid reversal of the direction of motion. The particles finally disappear from the field of view after a rapid transit along the centre of the channel.

When the mean velocity is increased to 10.3 cm. per sec. the flow form is two-dimensional and remains symmetrical (Fig. 4). The main flow now extends throughout the full length of the divergent section of the channel and separates the return flow regions. These regions occupy much smaller areas but the particles in them still move in the same characteristic way as described above. The flow leaves the wall at two corresponding points and moves in accordance with the predictions of Blasius.

When the mean velocity of 17.0 cm. per sec. is attained the flow form is unsymmetrical but remains two-dimensional (Fig. 5). The points at which the main flow leaves the walls no longer correspond and the flow deviates toward one wall. One of the return flow areas now becomes smaller and the other increases, though the particles in these regions still retain their characteristic motion.

At the maximum mean velocity of 22.5 cm. per sec. the flow is still two-dimensional but its unsymmetrical form is more pronounced (Fig. 6). The main flow breaks away from one wall and moves parallel to the other. A single reversed flow region exists in which the particles circulate as previously described.

The transitions of the flow forms from one to the other as the mean velocity is slowly increased through the range $0 \leqslant \bar{u} \leqslant 22.5$ cm. per sec. are gradual. At the lowest velocities the flow form, while preserving its symmetry and three-dimensional quality as shown in Fig. 3, changes continuously with the mean velocity. At first the bright area in Fig. 3 is small and extends outward only a short distance from the throat of the channel. With increase of mean velocity this area extends further out into the channel, while the reversed flow regions shift forward to a new position so that the general form remains the same. When a value $\bar{u}=7.5$ cm. per sec. is reached



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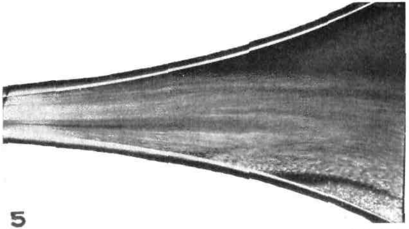

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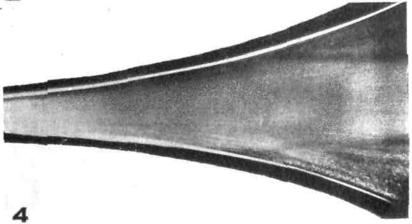

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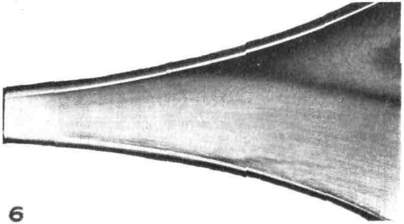

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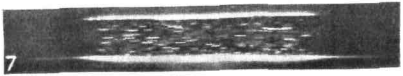

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. The three-dimensional symmetrical type of flow form at $R=18.7$, $\bar{u}=5.6$ cm. per sec. FIG. 4. The Blasius flow form at $R=31.4$, $\bar{u}=10.3$ cm. per ... 5. The two-dimensional unsymmetrical type of flow form in the transitional stage at $R=56.7$, $\bar{u}=17.0$ cm. per sec. FIG. 6. The two-dimensional unsymmetrical type of flow at $R=75.1$, $\bar{u}=22.5$ cm. per sec., in its completed form at the end of the transition. FIG. 7. Particle tracks in the region $x=0$ which are typical of those from which measurements of the initial velocity distributions were made. Time of exposure, 0.0355 sec.

the main flow extends across the field of view (Fig. 4) and the motion becomes two-dimensional. The flow form is now that described by Blasius. As the mean velocity continues to rise the Blasius flow persists for a time and then begins a very gradual transition at $\bar{u}=10.8$ cm. per sec. to that shown in Fig. 5. When observed in a large number of experimental trials it was found that the flow at this stage did not deviate toward the same wall in all cases, for during some of the trials the flow was observed to deviate to the left whereas in other cases the deviation was to the right. However, in any single trial, once the deviation toward one of the walls is begun, this deviation increases continuously as $\bar{u}$ is raised, until at $\bar{u}=21.0$ cm. per sec. the flow breaks away from one wall only. This flow continues with little change until the value $\bar{u}=22.5$ cm. per sec. is attained (Fig. 6).

Therefore two transitions occur: (1) the three-dimensional flow at low velocities becomes a two-dimensional flow at high velocities; (2) the symmetrical flow at low velocities changes to an unsymmetrical flow at high velocities.

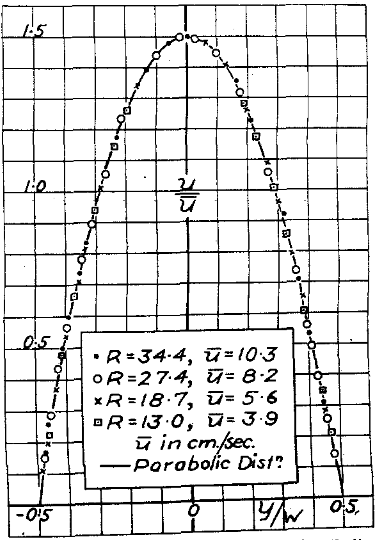

FIG. 8. *Experimental velocity distributions along the line $x=0$ at various values of $\bar{u}$ in the range $0<\bar{u}\leqslant 10.8$ cm. per sec. showing the existence of a laminar flow.*

A typical photograph of particle tracks from which measurements of the velocity distribution at $x=0$ were made is shown in Fig. 7. Representative points from the curves of velocity distribution obtained at $x=0$ for values of $\bar{u}$ of 3.9, 5.6, 8.2, and 10.3 cm. per sec. are plotted in Fig. 8. These values of $\bar{u}$ lie in the range $(0\leqslant\bar{u}\leqslant 10.8$ cm. per sec.) in which the symmetrical type of flow form was observed (Figs. 3 and 4). Values of $u/\bar{u}$ are plotted against y/w, where u is the velocity at a point distant y from the axis of the channel, $\bar{u}$ is the mean velocity and w is the dis-

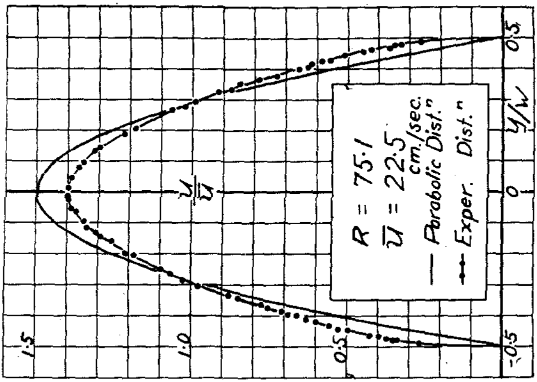

FIG. 10. The experimental curve of velocity distribution at $\bar{u} = 22.5$ cm. per sec. for the line $x = 0$ indicating that the deviation from the laminar flow increases as $\bar{u}$ rises.

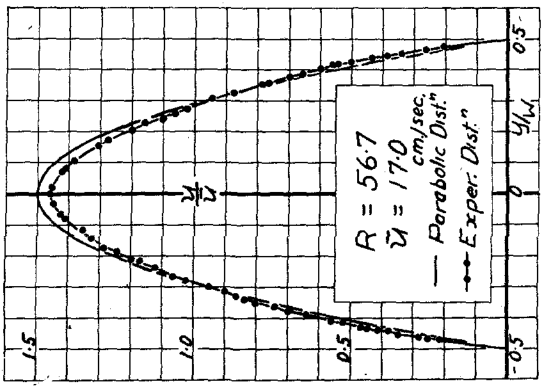

FIG. 9. The experimental curve of velocity distribution at $\bar{u} = 17.0$ cm. per sec. for the line $x = 0$ showing the deviation of the flow from the laminar type.

tance between the channel walls at $x=0$ (0.5 cm.). For comparison the parabolic distribution curve given by

$$\frac{u}{\bar{u}}=\frac{3}{2}\left[1-4\left(\frac{y}{w}\right)^2\right] \tag{2}$$

is also shown. It will be seen that the experimental points lie on the parabolic curve, and hence that the velocity distribution in the throat of the channel is parabolic in the range $0 \leqslant \bar{u} \leqslant 10.8$ cm. per sec.

The velocity distributions at $x=0$ for two values of $\bar{u}$ (corresponding to Figs. 5 and 6) in the range ($10.8 \leqslant \bar{u} \leqslant 22.5$ cm. per sec.) in which the unsymmetrical flow form was found are indicated in Figs. 9 and 10. A comparison with the parabola shows that the initial velocity distribution has now changed and the deviation from the parabolic form becomes greater as $\bar{u}$ increases. The curves in Figs. 8–10 are obtained at values of $\bar{u}$ corresponding to those of the flow forms shown in Figs. 3–6.

Discussion of Results

The experimental curve of velocity distribution in Fig. 10 is represented approximately by the empirical equation

$$\frac{u}{\bar{u}} = 1.40 - 8.48\left(\frac{y}{w}\right)^2 + 54.72\left(\frac{y}{w}\right)^4 - 172.4\left(\frac{y}{w}\right)^6 . \tag{3}$$

This is a particular form of the general expansion

$$\frac{u}{\bar{u}} = \Sigma a_n\left(\frac{y}{w}\right)^n , \qquad (n=0,2,4,\ldots) , \tag{4}$$

which, when evaluated to a sufficient number of terms, may be considered to give good agreement with the experimental velocity distributions shown in Figs. 9 and 10.

From Equation (3) it will be seen that, for small values of y/w the equation is approximately parabolic. Therefore, even at the highest velocities observed, the motion in the centre of the channel at $x=0$ is still essentially laminar. However, at large values of y/w the curve deviates quite appreciably from the parabolic form. The flow in those regions near the walls in the throat of the channel is no longer laminar. The increase of mean velocity to 22.5 cm. per sec. has therefore brought little change in conditions near the axis of the channel at $x=0$, but it has produced a non-laminar flow in the neighborhood of the walls at $x=0$.

The variation of velocity with the distance from the wall (4) as obtained from Figs. 8–10 in the range $0.4 \leqslant y/w \leqslant 0.5$ may be given in the form

$$\frac{u}{\bar{u}} = k\left(\frac{h-y}{w}\right)^n , \tag{5}$$

where $h=1/2w$. For a laminar flow $n=1$ and the conditions near the walls as shown in Fig. 8 are given by

$$\frac{u}{\bar{u}} = 5.70\left(\frac{h-y}{w}\right) . \tag{6}$$

The non-laminar distribution at the walls indicated in Fig. 9 may be written

$$\frac{u}{\bar{u}} = 4.00\left(\frac{h-y}{w}\right)^{0.802} . \tag{7}$$

Finally for $\bar{u} = 22.5$ cm. per sec. (Fig. 10) the velocity varies with the distance from the wall in a manner given by

$$\frac{u}{\bar{u}} = 1.77\left(\frac{h-y}{w}\right)^{0.423} . \tag{8}$$

These values of n are plotted against the corresponding values of the Reynolds number*, R, in Fig. 11. The smooth continuous curve drawn through the points indicates that n begins to decrease from the constant value 1 in the neighborhood of $R = 36$ ($\bar{u} = 10.8$ cm. per sec.) and continues to decrease as R increases above this value.

We may now discuss the flow forms in their relations with n. As the mean velocity is increased so that R passes through the range $0 \leqslant R \leqslant 36$ the value of n remains constant and equal to 1. Thus the flow at $x = 0$ in this range is laminar. The three-dimensional symmetrical flow (Fig. 3) gradually changes until at $R = 25$ ($\bar{u} = 7.5$ cm. per sec.) it transforms into the Blasius flow (Fig. 4) which persists until a Reynolds number of 36 is reached. During this transformation there has been no change of the initial velocity distribution. We may therefore consider the Blasius flow form to be the limiting case, as $\bar{u}$ increases, of a more general three-dimensional flow.

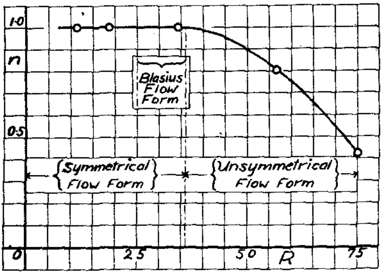

FIG. 11. *The variation of n with R showing the relation between the flow form and the type of motion near the walls of the channel at x = 0.*

The work of Kármán (4) and Blasius (2) shows that for a turbulent flow $n = 1/7$. It will be seen therefore that the transition from a laminar to a turbulent flow in the throat of the channel begins at $R = 36$. But simultaneously with the beginning of this transition the symmetrical flow form begins to lose its symmetry and the flow tends to deviate toward one wall. This deviation increases as n decreases (Fig. 5) until at a value $R = 70$ ($\bar{u} = 21.0$ cm. per sec.) the flow breaks away from one wall only (Fig. 6). This flow form was found to persist with little change up to $R = 75.1$ where, since $n = 0.423$, the transition to the turbulent flow is still incomplete.

* *The Reynolds number used here is defined by the relation* $R = \frac{\rho w \bar{u}}{\mu}$, *where* w (= 0.5 *cm.*) *is the width of the channel where the divergence begins,* $\bar{u}$ *is the mean velocity of flow calculated for the central horizontal plane of the channel at the line* $x = 0$, *and* ρ, μ *are respectively the density and viscosity of air at 23° C.*

In conclusion we may say: (1) the Blasius flow form is the limiting case, as $\bar{u}$ increases, of a more general three-dimensional flow and it exists in the range $25 \leqslant R \leqslant 36$; (2) the symmetrical Blasius flow form transforms, as R increases, to an unsymmetrical flow which breaks away from one wall only, the transition taking place over the range $36 \leqslant R \leqslant 70$; (3) at the point where the transition from the symmetrical to the unsymmetrical flow begins ($R = 36$), the flow near the walls at $x = 0$ ceases to be laminar and begins a transition to a turbulent flow which progresses as R increases.

Acknowledgments

The author wishes to thank Prof. E. F. Burton, Director of the McLennan Laboratory, and Prof. H. A. McTaggart for their interest and co-operation in this work. The author desires to express his appreciation of the assistance of Prof. K. B. Jackson of the Faculty of Applied Science and Engineering, University of Toronto, who placed at the author's disposal the equipment necessary for the calibration of the camera shutter speeds.

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A DISCHARGE TUBE LIGHT SOURCE WITH CONTROL CHARACTERISTICS SIMILAR TO THOSE OF A THYRATRON[1]

By D. C. Rose[2]

Abstract

Discharge tubes having electrical characteristics similar to those of thyratrons have been used as flashing light sources for recording purposes. The tubes differ from ordinary thyratrons in that the space between the control electrode and anode has been increased, so that the light given out by the intense discharge in this space is available. Two different tubes have been constructed. In the first the anode was placed in a side tube connected to the main tube by a capillary. The discharge through the capillary was used as the light source. An additional electrode was used in the main tube to start the discharge. In the second tube the space between the control electrode and the anode was enclosed by a cylindrical shield having a slit cut in its side through which the light from the discharge passed. These tubes were used successfully for recording the instant that beams of light were interrupted by rifle bullets in a new velocity-measuring apparatus. The tubes have the advantage over usual discharge tube light sources that they are as easily controlled as a thyratron. Other applications of such tubes as light sources are discussed.

The thyratron, though developed primarily as a relay device, has also been found to have uses as a light source for stroboscopic and recording purposes. Robertson and Rogers (3) and Kluge (2) describe the use of thyratrons as light sources for stroboscopic work. In measuring the velocities of projectiles the author (4) has used the light from an FG 17 thyratron to record the time of their flight. The flashes of light from the thyratron, as it discharged a condenser, were recorded photographically, the flashes being produced when the bullet interrupted beams of light placed along the trajectory. Other applications, such as the photographic recording for high speed counters of various sorts or any work where short time intervals between phenomena must be known accurately, suggest themselves.

Thyratrons, as usually designed, have the great disadvantage that the most intense part of the discharge takes place in a region which is so nearly enclosed by the electrodes that very little light escapes and what does is usually unduly diffused throughout the tube. An extended or diffuse light source may be useful for stroboscopic work, but for the recording purposes such as that required in the measurement of projectile velocities (4), or other cases where short time intervals between irregularly spaced and non-repeatable phenomena are being measured, the light source should be very intense and concentrated in a small area approaching a line or point source.

The discharge tubes described in this paper have the above-mentioned properties combined with the ease of control of an ordinary thyratron. In principle they are designed like a thyratron but the electrodes and glass envelope are so arranged as to emit as much light as possible but to confine it to a narrow line source. Edgerton and Germeshausen (1) have used for

[1] *Manuscript received September 26, 1934.*
Contribution from the Division of Physics, National Research Laboratories, Ottawa, Canada.
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stroboscopic work a controlled mercury discharge tube which could be used for purposes similar to those for which the tubes built by the author were used, but their tube suffers from the disadvantage that a high voltage, and therefore considerable energy from the control circuit, is required to start the discharge.

Description of Tubes

First Tube

Fig. 1 shows a diagram of the first tube tried, the important dimensions being given. The anode of the tube was located in a side tube connected to the main tube by a capillary. The capillary tube was used as the light source. The cathode consisted of an oxide-coated nickel strip about 1.5 mm. wide, 0.1 mm. thick and about 60 mm. effective length. The oxide coating was prepared from barium carbonate applied by the candle method and sensitized in the usual way. It would emit sufficient electrons to carry a continuous, current of almost two amperes, though of course the other electrodes would not stand this current for any length of time. A ring electrode was placed between the openings in the control electrode and the capillary tube, for the purpose of starting the discharge, so that unreasonably high voltages would not be required on the anode placed in the side tube far from the cathode and control electrode. If the ring electrode is used as an anode, the breakdown characteristics of the tube are similar to those of ordinary thyratrons of about the same size. The impedance of the capillary was such that 400 or 500 volts were required on the anode to start a visible discharge through it even when the main part of the tube was in a conducting state, *i.e.*, when current was flowing from the ring to the cathode.

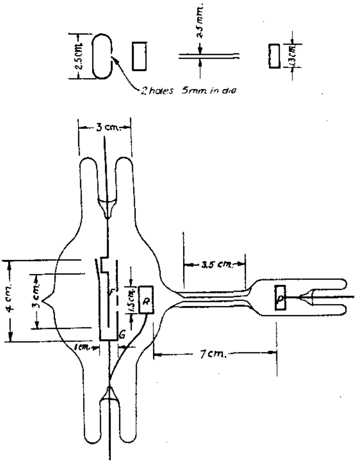

FIG. 1. *Arrangement of electrodes in the first tube.*

The tube was not designed for operation with continuous currents to the anode. If any appreciable current was made to flow continuously through the capillary tube it became dangerously hot. Hence for the most part its breakdown characteristics were studied while it was connected in a relaxation

oscillator circuit. The most satisfactory way to connect the ring to the power supply was that shown in the inset in Fig. 2. Under operating conditions the control electrode was maintained at a potential sufficiently negative that no discharge took place. Making it more positive than values given by the curves in Fig. 2 caused the tube to oscillate; or a short positive pulse on the control electrode would cause one discharge giving one flash of light. To obtain these curves the leads marked control were short-circuited and a voltmeter was connected from the cathode to the control electrode. The plate and ring voltages, as shown in Fig. 2, were calculated from the drop in the resistances in the plate circuit with the tube in a non-conducting state, the voltmeter being connected across the power input as indicated. The curves indicate the points at which the grid was just sufficiently positive to allow oscillation. Observations could be repeated to within 0.1 volt. The temperatures indicated are the temperatures of the air in a heated box surrounding the tube. Comparison of the curves in Fig. 2 with characteristic curves obtained when the ring was used as an anode shows that, over the range of voltages used, the discharge was controlled by the ring, and grid or control electrode. The anode voltages used were not high enough to make the tube conduct unless the discharge was started in the main part of the tube by appropriate voltages on the ring and control electrodes. In fact the ring would serve equally well for a control electrode but of course was not nearly as sensitive as the grid.

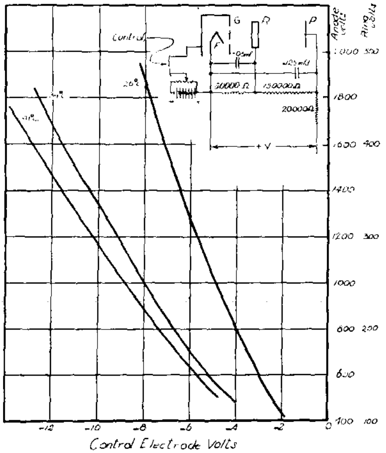

FIG. 2. *Discharge characteristics and circuit diagram for the first tube.*

As previously mentioned (4) the tube was used as part of a recording device for measuring the velocities of projectiles. The projectile (a rifle bullet) interrupted successively beams of light shining on photoelectric cells placed along the trajectory. The potential pulses, caused by the momentary change in light intensity, after being amplified, were impressed on the grid of the light-emitting thyratron. Fig. 3 shows a record obtained with the tube described above. The time marker on this record was made by an Einthoven galvanometer excited by an alternating current of 1000 cycles from a valve-main-

tained tuning fork. Only the peaks of the galvanometer fibre oscillations were included on the record. Under more complete working conditions the time record would be obtained from another similar tube flashing 1000 to 500 times per second and controlled by the 1000 cycle voltage. As only one tube of this type was constructed the time record and bullet record of the same type could not be taken simultaneously. This tube would not oscillate at 1000 cycles per second with the values of resistances and condensers used, and it is doubtful whether it could be made to oscillate at that frequency without cutting down the light intensity considerably. However it oscillated steadily at 500, $333\frac{1}{3}$ or 250 cycles per second when the 1000 cycle control was used, the flashing frequency depending on the grid bias.

A spectroscopic analysis of the light emitted from the capillary was not made though it would be an interesting study, as the color of the light appeared to be different from that of the light from an ordinary mercury discharge. The light appeared distinctly more yellow as though the yellow and green lines were enhanced at the expense of the blue and violet parts of the mercury spectrum. Bright yellow specks also appeared on the walls of the capillary as though minute points were made incandescent. One would expect that the number of excited atoms per unit volume in the capillary tube would be higher than in a less constricted discharge, owing to the high electron density in the constricted tube. However, until a more quantitative study of the light emitted at different pressures and current densities is made, it is difficult to say anything about the state of excitation of the mercury in the capillary tube. When the tube was used for recording, the anode voltage (+V, Fig. 2) was usually about 1500.

The period of the flash was extremely short, as shown by the line on the record. The lens system of the camera consisted of one cylindrical lens having its axis parallel to that of the capillary tube. The lens had an aperture (normal to its axis) of about 10 mm. and a focal length of 15 mm. The tube was placed about 25 cm. from the lens and the line image of the capillary was focused on the film in the rotating drum camera. With film speeds of about 5 mm. in 1/1000 second the record on the film did not appear any wider than the stationary

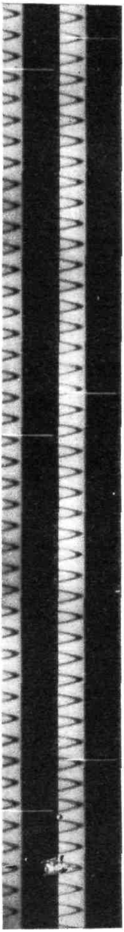

FIG. 3. *Record showing flashes of light from the first tube and time marker in apparatus for measuring projectile velocity.*

image of the capillary tube, and it was sharp on both edges, indicating that the time of discharge was not greater than a few micro seconds, probably considerably less than 10. Eastman Verichrome films were used in taking these records.

Second Tube

The second tube was designed with a view to decreasing the cathode to anode impedance so that lower anode voltages could be used. It was only partially satisfactory in this respect, but it has advantages over the first in that it gives more light, or more actinic light, than the first. A diagram of the tube is shown in Fig. 4. The arrangement is very similar to that of the

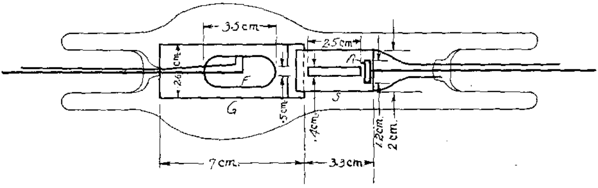

FIG. 4. *Arrangement of electrodes in the second tube.*

FG 17 thyratron, except that the distance between the grid and the anode was much greater and the space between them was enclosed by a cylindrical shield. This shield was slit on one side so that a narrow band of light was emitted. The shield was connected to the anode through a resistance of 25,000 ohms as shown in the inset circuit diagram in Fig. 5. To prevent the shield from carrying any of the discharge it should have been connected to the cathode. However, when it was connected to the cathode, the shielding effect was so great that the tube could not be made to discharge to the anode with the highest voltage available, namely, 2400 volts; and, with the shield connected as shown in Fig. 5, it appeared to carry a negligible part of the discharge and the tube worked very satisfactorily at 1000 volts. The cathode was prepared in a manner similar to that

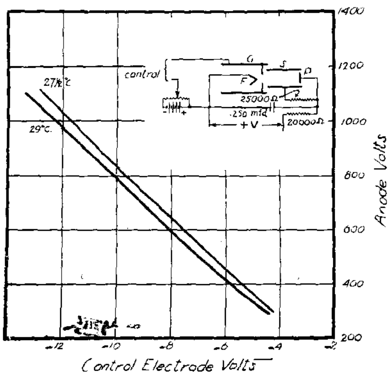

FIG. 5. *Discharge characteristics and circuit diagram for the second tube.*

in which the cathode of the first tube was prepared, but the electron-emitting area was about double. The curves in Fig. 5 represent the breakdown characteristics of this tube.

Fig. 6 shows records taken with the second tube used both to record interruptions of a light beam as in Fig. 3 and as a time marker emitting 500 flashes per second. Actually, the rifle range was not in operating condition when this tube was being tested, so a light beam was interrupted approximately 60 times per second by a cross arm bolted to the pulley of an electric motor. The light beam excited a photoelectric cell and the current pulses caused by the interruptions were made to control the same amplifier used in the projectile-velocity measurements. The period of the flash appears to be just as short as in the case of the first tube. The intensity of the light was considerably greater than that emitted by the first tube, but the two should not be compared as the energy of the discharge was considerably greater in the second case. Further, the discharge appeared different. In the second tube the discharge appeared to be the color of that in an ordinary thyratron.

Discussion

To make an efficient light source in a discharge tube, obviously a maximum number of atoms of the gas or vapor should be maintained in an ionized or excited state. In the case of a mercury arc where the hot pool of mercury at the cathode acts as an unlimited source of ions, the light intensity is limited only by the amount of energy that can be put into the discharge. However, such is not the case with tubes functioning as thyratrons where the main part of the discharge is carried by electrons emitted by a hot cathode. In fact, in using the thyratron as a relay device, satisfactory operation is achieved when the ionization is just sufficient to neutralize the space charge.

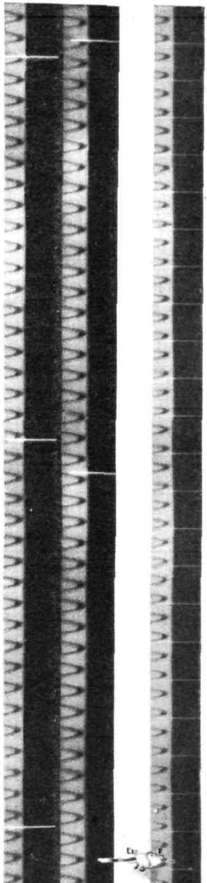

FIG. 6 *Record showing flashes of light from the second tube, used in the apparatus for measuring projectile velocity, and also used as a time marker giving 500 flashes per second.*

The amount of current carried by the positive ions in a thyratron is supposed to be small. When the tube is in a conducting state, voltages across the tube must be kept sufficiently low that positive ion bombardment of the cathode does not lower its efficiency as a source of electrons. The desired conditions for the thyratron as a relay and as a light source are opposite extremes, since in the ideal tube used as a relay the energy loss should be a minimum, while, as a light source, as much energy as possible should be transferred into light.

The amount of light emitted by such a tube will depend on the electron-emitting power of the filament, the pressure of the mercury vapor, the voltage across the tube when in a conducting state if used on continuous discharge, and both voltage and capacity of the condenser if used in a relaxation oscillator circuit. It will also depend on the nature and geometric arrangement of the electrodes, as is indicated by the different appearance of the light emitted by the two tubes discussed above. The size of the tube, arrangement of electrodes, etc., will be fixed to some extent by the purpose for which the tube is required, but to find the conditions for maximum light emission a careful spectroscopic and photometric study of the variation in the light intensity as a function of the above variables should be made. Like most electric lights the characteristics are probably such that the life of the tube will be shortened as its efficiency as a light source is increased, but experience with the two tubes constructed indicate that a reasonable compromise between efficiency and long life should be obtainable.

When the best conditions have been found, there is no reason why large tubes should not be built to give very intense light flashes for stroboscopic work, motion picture photography or any purpose where an easily controlled flashing lamp is desired. The part of the tube carrying the discharge could be of almost any shape.

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REVIEWS AND NOTES

Two Methods of Use when Dealing with Embedded Tissues

Several methods are in use for making temporary containers for liquid paraffin wax while embedding tissues, but most of them suffer from disadvantages in the matter of slow cooling, and in the amount of time expended in making special sizes.

The writer has obviated these difficulties in his own work by the use of hollow blocks of ice, made as follows.

Making Hollow Blocks of Ice

If a considerable number of blocks are required, and if the size to be used is appropriate, blocks may be made by filling the ice trays of a refrigerator with water, and allowing the water to chill until a substantial wall is built up around each "cube" and then pouring out the unfrozen centres. These hollow blocks will keep indefinitely in the refrigerator.

If blocks of special size are required, or if only a single one is needed, and none are on hand in the refrigerator, they may be made by melting out the centre of a piece of ice of appropriate size by means of a fine stream of hot water from an ordinary wash bottle, or even from the hot water tap.

Embedding in Hollow Blocks of Ice.

When embedding in these ice blocks, no special precautions need be taken, except to see that the paraffin is not so cool that it sets throughout its volume immediately on pouring into the ice. It will be found that a thick wall forms around the paraffin on pouring, and this provides a convenient floor for orienting the tissues. The cooling is extremely rapid, and as a result, the paraffin is free from all traces of crystallization, and cuts very well. Moreover, the blocks of paraffin separate cleanly and easily from the ice, owing to the melting of the latter. Of course, as soon as a skin forms over the paraffin, the whole should be thrown into ice water to hasten cooling still further.

Separating Portions of a Paraffin Block

The usual method of separating portions of a block of paraffin containing embedded tissues is to score the block with some sharp instrument, and then attempt to break it along the scored lines. This is not always successful, as the block may break along some other line, ruining tissues in the line of fracture.

This difficulty may be obviated as follows: a thin wire, such as the 1 string of a mandolin, or one strand of a woven-steel picture wire, is attache at one end to a convenient nail on the laboratory wall, and the other en allowed to hang free.

When it is desired to cut a piece out of a block, the wire is stretched tigh horizontally, and the paraffin block rubbed to and fro along it so that the wir saws its way through the wax. The cut may be guided in any direction depending on how the paraffin block is held. In this way, pieces may b cut from crowded blocks with no risk of damage to any part of the embedde tissues.

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